EASY TO REMEMBER, DIFFICULT TO FORGET:

THE DEVELOPMENT AND ENHANCEMENT OF FEAR REGULATION

A Dissertation

Presented to the Faculty of the Weill Cornell Graduate School

of Medical Sciences

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

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May 2015

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THE DEVELOPMENT AND ENHANCEMENT OF FEAR REGULATION

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Cornell University 2015

Fear extinction learning is a highly adaptive process that involves the integrity of frontolimbic circuitry. Its disruption has been associated with emotional dysregulation in stress and anxiety disorders. The most common behavioral approach to treating stress and anxiety disorders is based on the principles of fear extinction learning, that of exposure therapy. Many individuals don't respond well to this therapeutic approach, however, and why some individuals respond favorably to exposure-based therapies, and others don't, is not well understood. This thesis seeks to consider how developmental and individual differences influence the capacity to regulate fear, as well as to test a behavioral method that leads to enhanced fear regulation (i.e., attenuation of fear memory). Chapter 1 provides an overview of the relevant humans and rodent literatures on individual and developmental differences in cued-fear regulation. Chapter 2 presents the first evidence for adolescent-specific diminished cued-fear extinction learning in humans, paralleling results previously observed only in rodents. In Chapter 3, a common single nucleotide polymorphism in fatty acid amide hydrolase (FAAH), encoding an enzyme that plays an important role in the endocannabinoid system, is highlighted for its role in altering cued-fear extinction learning. Chapter 4 tests a novel behavioral method for enhancing cued-fear regulation based on the principles of memory reconsolidation in adolescent and adult

humans. Collectively, these studies point to markers that could potentially be used to identify patients for whom exposure therapy may not be effective and suggests an alternative approach that could lead to more efficacious treatments for these individuals.

BIOGRAPHICAL SKETCH

David Johnson was born and raised in Racine, Wisconsin. Prior to becoming interested in science, Dave spent 15 years as a professional live and studio musician in New York City. His passion for cognitive neuroscience was born in the laboratory of Dr. Elizabeth Phelps at NYU, where Dave earned his bachelor's degree in psychology. These interests were further cultivated during a two-year stint as a research assistant and lab manager in the psychology department of Harvard University under the tutelage of Dr. Jason Mitchell. Dave arrived at Weill Graduate School of Biomedical Sciences of Cornell University in 2010 and began conducting research in the laboratory of Dr. BJ Casey. Dr. Casey's stellar guidance and unwavering support have been crucial to the development and completion of this PhD thesis.

ACKNOWLEDGMENTS

BJ Casey: A very special thank you for your guidance, support and encouragement

Anjali Rajadhyaksha, Barry Kosofsky and Francis Lee:

Thank you for your valuable feedback and insight

Thanks to all of my current and former colleagues at the

Sackler Institute for Developmental Psychobiology

Financial support for this thesis was provided by the following grants:

P50MH079513 to BJC and 5T32EY007138 to DCJ

This dissertation could not have been completed without the emotional support provided

by Maddy S. Johnson, mom and dad, the dearly departed dog Marley

and the cats Mojo and Biscuit.

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Chapter 1

The development and enhancement of fear regulation: an introduction

Learning the relationship between threatening events and the cues that predict the onset of those events is an adaptive process that allows an individual to anticipate and minimize exposure to danger (Ohman and Mineka, 2001). Fear acquisition, the capacity to learn information about cues predictive of the onset of danger, is crucial to an individual's survival. By learning to identify cues that signal imminent danger, an individual can act on the presence of such cues to initiate a defensive response. However, the relationship between cues and consequences often changes over time. Thus it is important for an individual to regulate cue-driven fear responding when the predictive value of a cue changes from danger to safety. This can be accomplished through fear extinction learning, a context sensitive process in which repeated presentations of a previously conditioned threat stimulus, unpaired with an aversive outcome, leads to a decrease in fear responding to that stimulus. Evolutionarily preserved through time and across biological species, fear acquisition and fear extinction learning processes provide an individual with the flexibility to survive and thrive in a constantly changing environment.

Fear learning processes have been the subject of intense neuroscientific scrutiny in recent years, as substantial evidence suggests that when these processes become dysregulated, this can lead to the emergence of psychopathology. Maladaptive fear responding lies at the root of numerous psychiatric disorders, such as stress and anxiety

disorders and post-traumatic stress disorder (PTSD). The personal and societal costs of these disorders are immense. Anxiety disorders affect about 40 million American adults in a given year **(**Kessler et al., 2005), creating significant negative impact on quality of life for victims, as well as an enormous economic burden of more than $35 billion spent annually on treatment and indirect costs of over $4 billion per year in lost productivity (Greenberg et al., 1999).

The most common evidence-based behavioral treatment of anxiety disorders is cognitive behavioral therapy (CBT) (Rothbaum and Davis, 2003). Exposure-based CBT is based on principles of fear extinction learning and involves identification of what triggers the anxiety followed by systematic desensitization (repeated exposure) to that trigger in the absence of threat (Myers & Davis et al., 2002; LeDoux, 2000). Unfortunately, individuals who show diminished fear extinction learning may not respond well to exposure-based therapy, with only a little over 50% of individuals with clinical anxiety responding positively to exposure-based CBT (Walkup et al., 2008). However, the individual differences driving this variability in treatment response have not been well characterized. Furthermore, there exists the paradoxical situation in which the most common behavioral treatment approach for anxiety and stress-related disorders (exposure-based CBT) is built on the same learning process that mediates these individual's vulnerability to psychopathology in the first place (diminished fear extinction learning). Therefore, this dissertation is focused around three core goals: (1) identify specific developmental and individual difference factors that impact fear regulatory capacity; (2) elucidate the neural substrates underlying these effects, through examination and synthesis of human and animal studies, and (3) leverage this information

to devise and test new methods of enhancing fear regulatory capacity that could lead to novel clinical approaches for individuals who may not respond well to exposure therapy.

In this introductory chapter, I will begin by providing some background on fear acquisition and extinction learning and discuss the core neural circuitry. I will then discuss the developmental trajectory of this circuit and present evidence that fear extinction learning is specifically diminished during the developmental stage of adolescence. I will next present evidence pointing to specific genetic factors (variants of the genes BDNF and FAAH) that play a role in fear regulation. I will outline a neural substrate for diminished fear regulation common across the causal factors of age, experience and genetic profile; that is, immature functional integrity in a neural circuit consisting primarily of the prefrontal cortex and the amygdala. Finally, I will focus on the clinical implications of diminished fear extinction learning and introduce alternatives to exposure-based methods of fear regulation, including a method based on the principles of memory reconsolidation that may bypass frontal regulation of fear and inform optimized clinical treatments for individuals with pathological anxiety who don't respond well to exposure therapy**.**

Fear Learning: an introduction

Fear learning is an adaptive process that allows an organism to respond appropriately to cues or contexts that predict danger. Behavioral paradigms based on the principles of classical conditioning have become the *de facto* standard for studying fear learning in animals and humans. Classical conditioning is a process based on Pavlovian learning principles in which a neutral stimulus is paired with a salient stimulus (Pavlov

and Anrep, 1927). During fear conditioning, a conditioned stimulus (the cue) is repeatedly paired with an aversive event (the unconditioned stimulus), such that the presentation of the cue alone comes to elicit a fear response, indicating the acquisition of a conditioned fear response (LeDoux, 2003). Once an associative link between the cue and aversive stimulus is formed and consolidated, it becomes a stable long-term memory.

After a cue is no longer predictive of the onset of danger, however, it is maladaptive to respond as if it is still a threat. Typically a conditioned fear response can be reduced by extinction. During extinction, the cue is repeatedly presented by itself and fear expression decreases, as the animal learns that it no longer reliably predicts the aversive stimulus (Mackintosh 1974). Early models of fear extinction learning posited that extinction involved the unlearning of associations between a cue and an aversive stimulus (Rescorla and Wagner, 1972). However, it is now accepted that extinction reflects learning of a new memory trace that competes with the original fear memory for expression (Bouton 2004; Myers and Davis, 2002). If the extinction memory is strong enough and can be successfully retrieved, fear expression can be suppressed. Substantial evidence shows, however, that while extinction learning can reduce the expression of conditioned fear, extinguished fear may return under a number of different circumstances including the simple passage of time (spontaneous recovery), exposure to an aversive stimulus or stressor (reinstatement) or exposure to a threat cue in a novel context (renewal) (Bouton 2004; Myers and Davis, 2002). In adaptive terms, this computes logically as the predictive value of an extinguished threat cue might become unclear under these conditions, and the penalty for failure to appropriately respond to a threat cue could be injury or death. The return of extinguished fear is therefore not categorically

maladaptive. However, when fear regulatory capacity is diminished an individual may respond repeatedly to cues once predictive of danger, even though danger is no longer present. Persistent fear responding to a safety cue is maladaptive and can lead to pathological states of anxiety.

The neural circuitry of fear acquisition and extinction learning

Substantial research in animals and humans has characterized the neural mechanisms underlying fear acquisition and fear extinction learning (Figure 1.1). The amygdala, a structure in the medial temporal lobe, is functionally segregated into subnuclei that play distinct roles in fear acquisition and expression (LeDoux 2007). During fear learning sensory thalamic inputs converge on the lateral amygdala (LA) (Quirk et al., 1995; Collins and Pare, 2000) driving fear expression through the central nucleus (CE) of the amygdala downstream toward output systems that mediate autonomic responses (Maren 2001). Learning has occurred when the conditioned stimulus alone is able to initiate activity in the LA and elicit a fear response, which prior to conditioning would have been elicited only by the unconditioned stimulus. The hippocampus also plays a significant role in expression of fear memories that go beyond this dissertation.

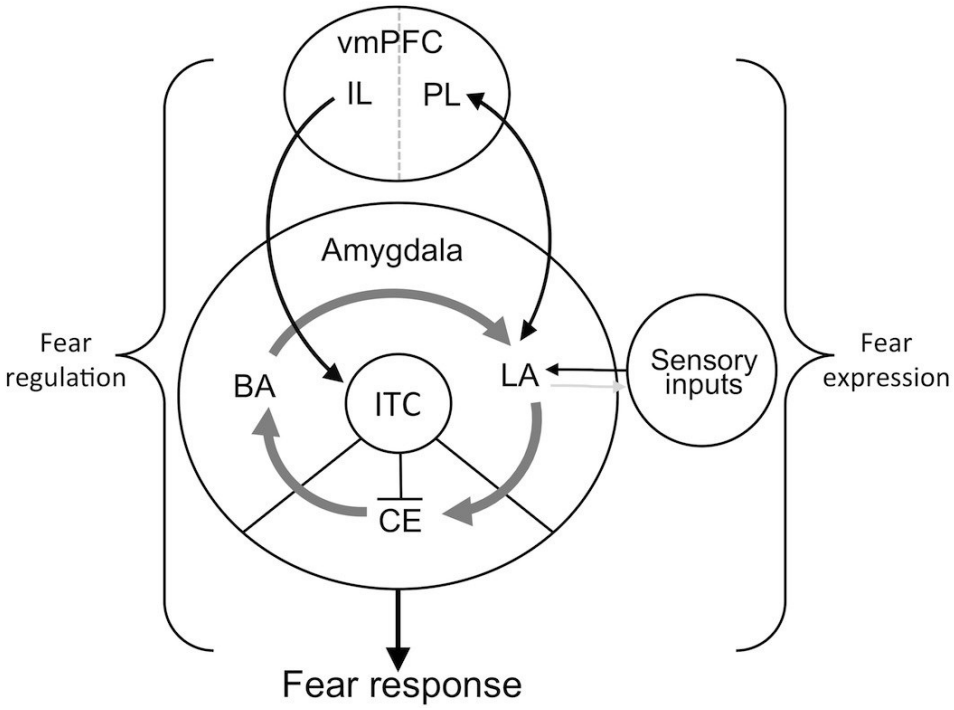

Figure 1.1 Cued-Fear Circuitry. A simplified diagram of the neural circuitry underlying regulation of threat-related cues in rodents. Abbreviations: IL, infralimbic prefrontal cortex; PL, prelimbic prefrontal cortex; BA, basal amygdala; LA, lateral amygdala; CE, central amygdala; ITC, intercalated cells; vmPFC, ventromedial prefrontal cortex. Human regions homologous to the rodent PL and IL are dACC and vmPFC, respectively (from Johnson & Casey, 2015a).

The ventral medial prefrontal cortex (vmPFC) is critical for mediating fear expression and extinction (Quirk and Mueller, 2008; Phelps et al., 2004). Two distinct subregions of the rodent vmPFC, the prelimbic and infralimbic cortices, play specific functional roles in the expression and inhibition of fear, respectively. (Santini et al., 2008; Sierra-Mercado et al., 2011; Sotres-Bayon and Quirk, 2010). The prelimbic cortex (PL) has been implicated in the expression of fear via bilateral projections to and from the amygdala (Milad and Quirk, 2012). The PL receives transient inputs signaling the presence of threat from the amygdala and transforms these signals into sustained firing via downward projections to the CE (Sotres-Bayon and Quirk, 2010) and toward output

systems that generate fear responses. The infralimbic cortex (IL) plays a contrasting role in the storage and recall of extinction memory (Quirk and Mueller, 2008). The LA and basal nucleus (BA) of the amygdala excite cells in the IL in response to safety signals (Repa 2001). Cells in the IL then modulate fear expression through projections to inhibitory (intercalated) cells in the amygdala, that in turn block activity in the CE, suppress outputs to downstream targets and blunt fear expression and related autonomic activity (Milad and Quirk, 2012). Thus, the vmPFC does not simply play an inhibitory role in fear regulation but rather regulates low and high fear states through subnetworks defined by bilateral projections between distinct regions of the vmPFC and functionally specific nuclei in the amygdala (Sotres-Bayon and Quirk, 2012).

Many studies have shown that fear circuitry is highly conserved across species, suggesting similar neural mechanisms underlie fear learning and extinction in both mice and humans (Soliman et al., 2010; Milad et al., 2007a; Gottfried and Dolan, 2004). Technical limitations make it challenging to precisely delineate regions homologous to rodent infralimbic and prelimbic cortex in the human brain (Milad and Quirk 2012). However, the dorsal anterior cingulate cortex (dACC) has been associated with expression of conditioned fear in humans and has been proposed as the human homologue of the rodent prelimbic cortex (Milad et al., 2007a). This is supported by fMRI BOLD data that has shown dACC activity increases with expression of conditioned (Milad et al., 2007a) and unconditioned fear (Dunsmoor et al., 2008). Numerous human studies have demonstrated the importance of the vmPFC in fear extinction learning, supporting this region as functionally and structurally homologous to the rodent infralimbic cortex. Functional imaging studies show that increased vmPFC activity is

associated with less fear expression during extinction learning (Phelps, 2004; Kalisch et al., 2006) and better recall of extinction memory (Milad et al., 2007b). MRI-based volumetric studies show that larger vmPFC volume is associated with greater fear extinction learning (Shin, et al 2006) and better retention of extinction memory (Milad et al., 2005).

Together these studies demonstrate that while the amygdala plays a role in acquisition, storage and retrieval of fear memory, regulation of fear is dependent on bilateral connections between the amygdala and the vmPFC. Converging evidence has linked anxiety and anxiety-related disorder to impaired fronto-amygdala regulation. Trait anxiety has been associated with heightened amygdala activity during fear learning in human adults (Indovina et al., 2011), while individuals with increased trait anxiety show compromised fear extinction learning that appears to be driven by dysreguated interactions between frontal and amygdala regions (Indovina et al., 2011; Lissek et al., 2005). In clinical populations, PTSD patients have shown diminished prefrontal blood flow in PET studies (Bremner et al., 1999; Semple et al, 1996), reduced vmPFC activity when recalling traumatic events (Shin et al., 1999) and impaired fear extinction learning (Milad et al., 2008). In sum, these data suggest diminished functional capacity in fronto-amygdala circuitry may underlie deficits in fear extinction learning and could be a factor contributing to the onset of pathological fear and anxiety.

Anxiety disorders peak during adolescence (Merikangas et al., 2010; Costello et al., 2005). These disorders often persist into adulthood and early onset is often predictive of the most severe and disabling forms of adult psychopathology (Andersen and Teicher,

2008; Kim-Cohen, et al., 2003). Adolescence is also a developmental stage during which functional immaturity of fronto-amygdala circuitry is observed with stronger connectivity between early-developing subcortical regions than in later-maturing prefrontal regions (Casey et al., 2008). Therefore, the PFC is less capable of sufficiently suppressing emotions and actions mediated by subcortical limbic structures during adolescence. In the next section, I review studies that collectively describe how functional immaturity in prefrontal-amygdala connections may underlie adolescent-specific diminished fear extinction learning.

Structural and functional changes in frontolmbic circuitry during adolescence

There are regional structural and functional changes in brain circuitry during adolescence. Nonhuman animal work and post mortem human studies show that synaptic pruning reaches adult numbers in sensorimotor cortices before the prefrontal regions (Bourgeois et al., 1994; Huttenlocher & Dabholkar 1997). These regional changes are paralleled by human developmental imaging studies that show peaks in cortical thickness and volume in sensorimotor cortices and subcortical regions before association cortices (Gogtay et al., 2004; Sowell et al., 1999, 2004; Mills et al., 2014). This pattern of development can result in a functional imbalance during adolescence characterized by high, subcortically-driven reactivity to emotional events and low capacity to regulate emotional responses to these events through prefrontal connections. Recent evidence (McCallum et al., 2010; Hare et al., 2008) suggests that immature top down ventromedial (infralimbic) prefrontal projections to the amygdala during adolescence may lead to diminished fear extinction learning.

An example of functionally altered frontolimbic activity during adolescence comes from a study that examined developmental changes in fMRI BOLD signal to threat-related cues (fearful faces) (Figure 1.2A). This study demonstrated heightened amygdala activity to threat cues in adolescents relative to both children and adults (Hare et al., 2008; Figure 1.2B), consistent with other work showing that adolescents exhibit greater amygdala responses to emotional pictures than adults (Guyer et al., 2008; Monk et al., 2003).

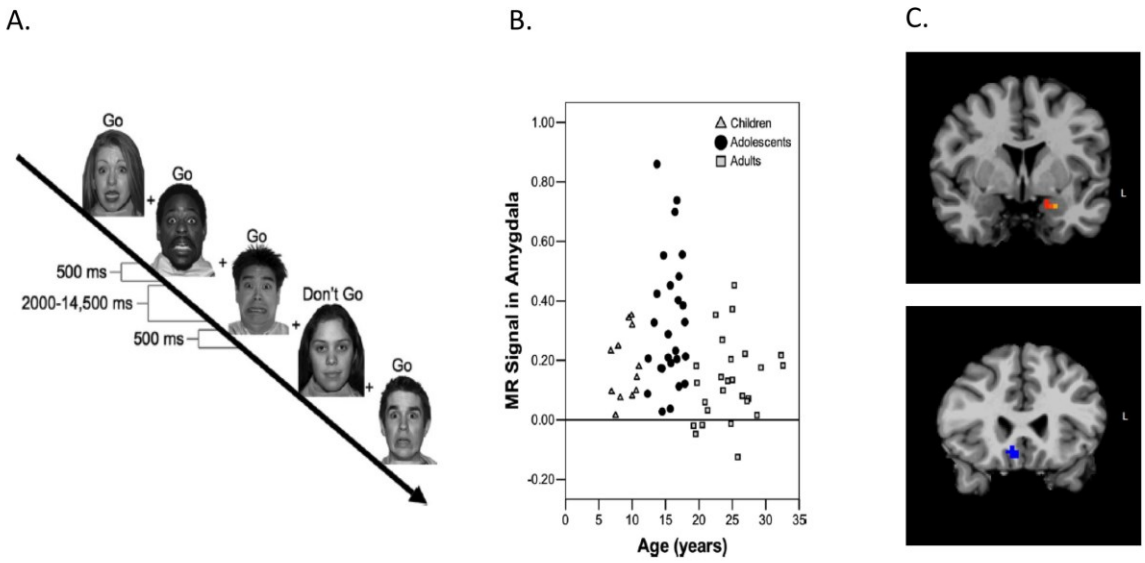

Figure 1.2. Adolescent-specific differences in threat reactivity and regulation. (A) Participants were instructed to either press or not press a button in response to neutral or angry faces. (B) Adolescents show greater amygdala reactivity to threat cues (angry faces) compared to children and adults. (C) Cortical and subcortical neural regions associated with reaction time for fear targets. Region of left amygdala showed positive correlation with reaction time (top panel). Region of vPFC showed negative correlation with reaction time (bottom panel) (from Hare et al., 2008).

In this study, threat cues generated behavioral inhibition, as measured by increased time to respond to threat relative to non-threat cues. Threat-related slowing in response latencies corresponded to greater amygdala and decreased vmPFC activity (Figure 1.2C). This inverse pattern between the vmPFC and amygdala is consistent with

the role of the prefrontal cortex in modulating and regulating the fear response via projections to inhibitory cells (intercalated cells) in the amygdala that in turn inhibit central nucleus output that dampens the fear response. To further constrain the interpretation of these findings, the researchers examined changes in amygdala activity as a function of time and to what extent habituation of the amygdala response over time was correlated with vmPFC activity. Greater habituation in the amygdala was associated with greater connectivity between vmPFC and amygdala, with less habituation in the amygdala response correlating with higher self-reported trait anxiety. These latter findings are consistent with studies showing that fear regulatory circuitry is functionally compromised in anxious individuals (Kim and Whalen, 2009). While this study provides support consistent with the hypothesis of diminished functional capacity of prefrontal regions to inhibit amygdala responses to threat-related cues, there are not as of yet any published studies that have directly tested this idea using associative (Pavlovian) learning paradigms in adolescent humans. For insight, we turn to human behavioral studies and rodent behavioral and neurobiological evidence that suggest adolescent-specific changes in fear regulation are mediated by a functional imbalance between prefrontal inhibitory regions and subcortical activity that drives fear expression.

Fear regulation across development

Although there is a large body of research examining fear extinction learning in adults, there is a surprising scarcity of research pertaining to adolescents. In one of the first rodent studies to directly test adolescent fear extinction learning, Kim and Richardson (2011) compared the behavior of pre-adolescent (p28), adolescent (p35) and

adult (p70) rats in a Pavlovian conditioning task. Although adolescent rodents showed within-session extinction equivalent to young adults and pre-adolescents, they showed attenuated extinction retention (enhanced return of fear 24 hours after extinction). In other words, despite no difference in attenuating fear during extinction, adolescents showed a significant return of fear later. This study also demonstrated insufficient recruitment of the infralimbic cortex during extinction in adolescent compared to adult and preadolescent rodents.

Pattwell et al. (2012a) have recently shown diminished within- and between-session extinction learning in adolescent mice. Adolescent mice (p29) showed increased freezing at various test points over multiple days of extinction training compared to both adults (p70) and pre-adolescents (p23). This pattern was paralleled by diminished synaptic plasticity in infralimbic cortex of the adolescent mice compared to the pre-adolescents and adults.

To date, there have been very few studies examining fear conditioning and extinction learning in human adolescents. In one study, Haddad and colleagues (2011) presented teens with photographs of neutral faces paired with a negative event (angry face plus a critical comment), a positive event (happy face plus a compliment) or neutral event (neutral face plus a neutral comment). After each phase of the experiment, participants rated each stimulus for "scariness." Participants rated the faces paired with negative outcomes as significantly more scary than those paired with positive or neutral outcomes after acquisition and extinction. Although this study demonstrated that adolescents were resistant to extinction it is difficult to attribute the findings to age as

there were no other age groups to which to compare the findings. Adolescent-specific diminished fear extinction learning ideally requires an adult and/or child group for comparison. In chapters 2 and 4, I will present data from two studies that contribute to filling this gap in the developmental fear learning literature, demonstrating diminished fear extinction learning in human adolescents compared to children and adults.

Genetic influences on fear regulatory processes

The general properties of fear learning systems and underlying neural substrates have been relatively well characterized over the past several decades (Phelps et al., 2004). However, factors that underlie individual variability in fear regulation are less well understood. While the developmental effects on fear extinction learning described in the aforementioned studies (Pattwell et al., 2012a; Johnson & Casey, 2015b) are robust on a population level, variability within both the adolescent and adult human samples highlight the importance of exploring factors that mediate individual differences in extinction learning processes.

Emerging evidence suggests that fear learning is heritable (Hettema et al., 2003). As the neural circuitry underlying fear learning and regulation is quite complex, it is reasonable to assume that heritable influences on these processes involve multiple genes, each of which (or combinations thereof) possessing at least partially distinct functional roles. In this section I focus on two genes that play a central role in regulating fear extinction learning: brain derived neurotrophic factor (BDNF), implicated in multiple learning and memory processes, and fatty acid amid hydrolase (FAAH), an enzyme proposed to play a central role in anxiety-related behavior.

Brain-derived neurotrophin factor (BDNF)

BDNF is a neurotrophin with a key role in mediating plasticity in the brain and regulating various learning and memory processes (Bramham and Messaoudi, 2005; Mahan et al., 2012), including fear extinction learning (Peters et al., 2010; Egan et al., 2003). In humans, the BDNF gene contains a single-nucleotide polymorphism (SNP) at codon 66 that leads to a valine to methionine substitution (val66met). The presence of this SNP leads to decreased activity-dependent release of BDNF (Egan et al., 2003). One of the behavioral consequences of this SNP was highlighted by a study showing increased anxiety-like behavior in genetically-modified Val66Met mice (with a methionine substitution at codon 66) compared to wild types (Chen et al., 2006), with the Val66Met mice spending less time in the open arms of a T-maze.

Because dysregulated fear expression is a fundamental component of anxiety-related behavior, Soliman et al. (2010) conducted a parallel study in mice and humans to test whether BDNF might exert an influence on fear extinction learning and associated neurobiological substrates (Soliman et al., 2010). In both mice and humans, the Met allele carriers showed less extinction learning than non-Met allele carriers (Figure 1.3A, 1.3B). Humans showed concordant alterations in fMRI BOLD activity in fear regulatory circuitry, with Met allele carriers showing less vmPFC and greater amygdala activity during extinction than non-Met allele carriers (Figures 1.3C, 1.3D).

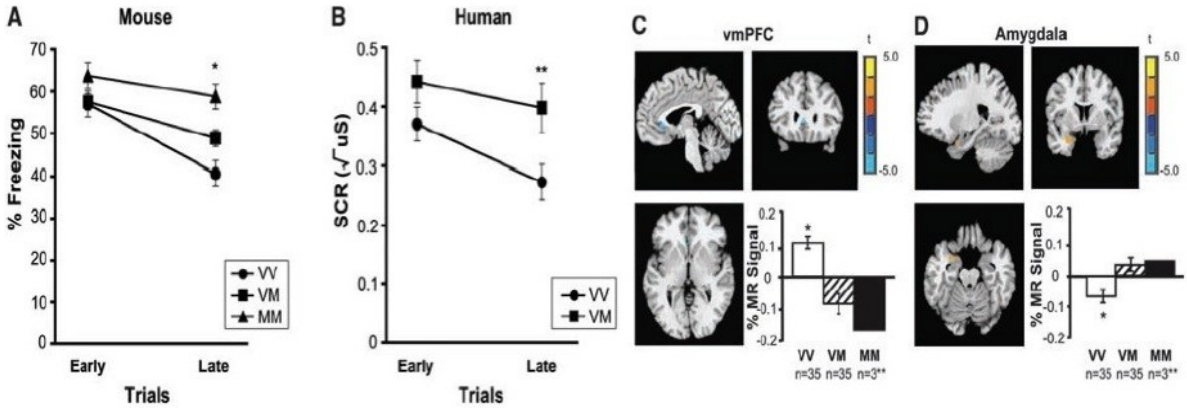

Figure 1.3 Altered extinction learning in mouse and human Met allele carriers.
(A) Diminished extinction in mice with BDNF Val66Met as indexed by changes in freezing across extinction. (B) Similar effects were shown in human Met allele carriers, as measured by changes in skin conductance response (SCR) during extinction. (C) Human Met allele carriers showed less BOLD activity in the vmPFC during extinction. (D) Humans with BDNF Val66Met showed increased amygdala activity compared to val/val homozygotes. (from Soliman et al., 2010).

Subsequent rodent studies have attributed this BDNF-mediated impairment in extinction learning to diminished synaptic plasticity in infralimbic cortex (Pattwell et al., 2012b). Decreased capacity to extinguish fear memories can be rescued by infusion of BDNF into this same region (Peters et al., 2010).

These findings are concordant with studies that have linked BDNF genotype to increased stress reactivity underlying posttraumatic stress disorder (PTSD), a pathological condition characterized by impaired fear extinction learning. Met carriers have an increased risk for PTSD compared to non-Met allele carriers (Rakofsky et al., 2012) with a 3-fold increase in carriers homozygous for the Met allele (Zhang et al., 2014). Together these findings underscore the importance of neurotrophin factor in learning when cues of potential danger are no longer a threat. These findings further

suggest that decreased available BDNF may play an important role in vulnerability to anxiety and stress-related disorders due to less capacity to regulate fears and emotions.

Fatty Acid Amide Hydrolase (FAAH)

The endocannabinoid system has been implicated as playing a role in anxiety and stress-related processes and disorders (Hariri et al., 2009; O. Gunduz-Cinar et al., 2013a**)**. Anandomide (AEA), an endogenous cannabinoid (eCB) that modulates synaptic transmission through stimulation of the CB1 receptor, has been suggested to play a key role in this effect (O. Gunduz-Cinar et al., 2013a; O. Gunduz-Cinar et al., 2013b). Fatty acid amide hydrolase (FAAH) is a catabolic enzyme that controls levels of AEA signaling in the brain (Cravatt et al., 2001). In humans, a SNP exists in FAAH that leads to decreased levels of FAAH, elevated AEA and enhanced signaling activity at CB1 receptors (Sipe et al., 2002; Sipe et al., 2010). Genetic or pharmacologic disruption of FAAH is associated with decreased anxiety-like behaviors (Kathuria et al., 2003; Moreira et al., 2008) and enhanced fear extinction learning (Gundaz-Cinar et al., 2013a; Kathuria et al., 2003: Chhatwal et al., 2005). However, the ability to characterize the effects of the FAAH variant in the brain has been limited, since the variant is only present in humans. In Chapter 4 I present evidence in support of a central role of FAAH for enhancing fear extinction learning in humans, along with supporting data from a knock-in mouse, enabling the demonstration of parallel molecular, circuit-level and behavioral phenotypes in humans and in the knock-in mice carrying this variant. This evidence suggests a central role for FAAH in mediating fear regulatory capacity.

Methods for enhancing extinction – pharmacological and behavioral

Together, the literature suggests developmental time points, as well as genetic factors, that may reduce the efficacy of exposure therapy for specific individuals. In these cases, alternative evidence-based treatments might be employed.

Pharmacological interventions offer one alternative. A pharmacological approach most commonly involves conducting extinction training in conjunction with the administration of a pharmacologic agent designed to increase plasticity in the neural circuitry underlying extinction learning and memory. Several drugs have been shown to enhance fear regulation. Administration of D-cycloserine (DCS) led to enhanced extinction retrieval in adolescent and adult rats (McCallum et al., 2010; Baker et al., 2012). DCS has also been shown to decrease fear in humans, with one study showing that DCS administered before virtual reality exposure therapy for acrophobia led to decreased fear at a subsequent fear recovery test. (Ressler et al., 2004). On the contrary, DCS has failed to enhance within-session extinction or extinction retrieval in other human studies (Klumpers et al., 2012; Guastella et al., 2007). Serotonin selective reuptake inhibitors (SSRIs) such as fluoxetine and citalopram have proven effective in enhancing extinction, with studies showing that chronic administration of SSRIs in combination with extinction training prevented the return of fear in mice (Karpova et al., 2011; Deschaux et al., 2011). Recent studies have also explored the role of dopamine in the consolidation of extinction memories. Methylphenidate, the dopamine and norepinephrine reuptake inhibitor known commercially as Ritalin, has been shown to enhance contextual extinction learning when administered before and during extinction training in mice

(Abraham et al., 2012). Haaker et al. (2013) showed that systemic administration of L-Dopa (the precursor of dopamine) after extinction led to a decrease in contextual renewal; that is, the context dependence of extinction learning was reduced, as were spontaneous recovery and reinstatement. All of the aforementioned drugs can be administered systemically and are approved for use in humans. It should be noted that no single neurobiological mechanism, neurotransmitter or receptor subtype is responsible for the pharmacologically-mediated enhancement of fear extinction, highlighting the large number of molecular entry points into the fear learning circuitry. Data from animal studies suggests additional pharmacological agents such as histone acetylation modulators (Lattal et a., 2007), fibroblast growth factor (Graham & Richardson 2009), neuropeptides (Lach and de Lima, 2013; Gutman et al., 2008; Verma et al., 2012) and M-type potassium channel modulators (Santini & Porter 2010) can enhance regulation of fear, but these drugs have, as of yet, only been tested pre-clinically in animals. It is important to note that off-target effects diminish the attractiveness of a pharmacological approach, as systemic administration of many of these drugs can produce undesirable acute side effects, while chronic effects, particularly in the developing brain, are not well understood. Furthermore, its not known if the effects of these drugs to enhance fear regulation is long lasting. Nonetheless, a pharmacological approach may be net beneficial for some individuals, particularly for those who don't respond well to non-pharmacological treatments. It is also important to note that not all individuals will respond equally well to any given drug. Individual differences in the neurophysiological profile of the fear circuitry suggests that specific drugs for specific patients, administered singularly or in combination with other drugs, would be most likely to maximize positive

treatment outcomes for any given individual, assuming valid and reliable assessment of individual response profiles can be made.

Rodent studies have demonstrated that adolescents can benefit from an increased number of exposure trials during extinction (McCallum et al., 2010). However, this approach may not be ideal for adolescents with anxiety disorders as it would require additional time and money and could lead to higher attrition rates and increased failure to complete treatment. One promising behavioral approach, described below and reported in Chapter 4 (Johnson & Casey, 2015b), is based on the principles of memory reconsolidation.

Memory Reconsolidation: A possible temporal window during which fear memories can be altered?

The traditional view of memory formation is that it involves a one-time consolidation process, after which a memory becomes stable and no longer prone to interference (Squire and Davis, 1981; McGaugh, 2000). Research has demonstrated that consolidation of a new long-term memory could be disrupted by blocking protein synthesis (Schafe et al., 1999) or pharmacological intervention (Pitman et al., 2002; Vaiva et al., 2003), but only if the intervention occurred shortly after training and not several hours later. This approach has limited clinical utility because it is difficult to get access to patients immediately following a traumatic event. Therefore, an increasing amount of recent interest has been focused on reconsolidation as a temporal target for interfering with or attenuating fear memories.

The memory reconsolidation hypothesis suggests that every time a memory is retrieved it becomes unstable (Sara 2010) and dependent on *de novo* protein and RNA synthesis for restabilization (Dudai 2006; Nader et al., 2000). The plasticity induced by memory retrieval opens up a "reconsolidation window" during which a memory becomes prone to disruption (Dudai, 2006). This plasticity has been demonstrated by findings in rats that showed fear memory erasure could be induced after post-retrieval intra-amygdala infusion of protein synthesis inhibitors anisomycin (Nader et al., 2000; Duvarci and Nader, 2004) and U0126 (Doyere et al., 2007), and NMDA receptor antagonist MK-801 (Lee et al., 2006). Unfortunately, these compounds are toxic and not safe for use in humans (Duvarci and Nader, 2004).

Dębiec and LeDoux (2004) showed that intra-amygdala and systemic infusion of beta-adrenergic receptor blocker propanalol (non-toxic and safe in humans) could disrupt fear memory reconsolidation in rats. This finding was recently extended to humans using systemic treatment (Kindt et al., 2009). In this study, participants who received propranolol prior to retrieval of a conditioned fear memory showed persistent attenuation of fear response on a subsequent fear recovery test (but see Schiller and Phelps, 2011). Another promising approach to attenuating fear memory during reconsolidation was recently tested by Graff et al (2014), with evidence suggesting epigenetic mechanisms can be targeted, in combination with extinction training, to permanently modify fear memories during reconsolidation in rodents. Even if pharmacological or epigenetic approaches proved safe in humans and effective in disrupting reconsolidation, a behavioral procedure would still be preferable due to possible off-target effects, assuming similar effects could be obtained with behavioral methods.

Such a procedure was introduced by Monfils et al (2009) and has recently been tested in human adults (Schiller et al., 2010a). This methodology capitalizes on reconsolidation theory not by interfering with reconsolidation processes, but rather by using behaviorally induced interference (post-retrieval extinction) to introduce new information about the conditioned stimulus during reconsolidation, update the original memory trace and prevent the return of fear (Oyarzun et al., 2012; Agren et al., 2012, and Steinfurth et al., 2014 but also see Kindt and Soeter, 2013 and Golkar et al., 2012).

This method involves activation of a fear memory by presentation of an isolated retrieval trial followed by an extinction session that takes place during the subsequent reconsolidation window. The precise temporal duration of this window is not precisely known but is thought to be from 10 minutes to up to at least an hour, but not more than 6 hours after retrieval (Monfils et al., 2009). Schiller et al (2010a) designed two experiments to test whether behavioral extinction training during memory reconsolidation would lead to persistent attenuation of fear. In the first study, three groups of participants were differentially conditioned to acquire fear by pairing a colored square (the conditioned stimulus) with an aversive reinforcer (an electric shock) while another colored square was never paired with the electric shock (experimental day 1). Twenty-four hours later (experimental day 2), participants underwent an extinction session during which both conditioned stimuli were presented repeatedly unpaired with the electric shock. All participants showed significant fear extinction, as indexed by decreases in skin conductance response (SCR) to the conditioned stimulus. In two groups, the conditioned stimulus was reminded prior to extinction via a single presentation trial. One group received the reminder trial 10 minutes before extinction, while the other group received

the reminder trial 6 hours before extinction. The third group did not receive a reminder trial prior to extinction. Another 24 hours later (experimental day 3), participants returned to test for spontaneous recovery of the conditioned fear response. Participants who weren't reminded of the conditioned stimulus prior to extinction or who were reminded but extinguished outside of the reconsolidation window showed robust recovery of fear for the conditioned stimuli, but there was no spontaneous recovery of fear in participants for whom the CS+ was reactivated 10 minutes prior to extinction. Attenuation of conditioned fear was still present when participants returned for a fear recovery test one year later, demonstrating the persistence of this effect (Schiller et al., 2010a).

In a second study, a within-subject design was employed to control for the possibility of unintended group effects and allow for direct comparison of the return of fear with or without reconsolidation update. Participants were conditioned to two different colored squares (conditioned stimuli) via partial reinforcement with electric shock, while a third square was never paired with the shock. Prior to extinction, one of the conditioned stimuli was reminded via a single presentation trial, while the other was not. Recovery of fear was elicited via four unsignaled presentations of the US prior to the fear recovery test (reinstatement), which has been demonstrated as a robust method to elicit the return of an extinguished fear memory (Bouton 2004). Participants only showed a reinstated fear response to the conditioned stimulus that was not reminded prior to extinction. These studies collectively support the notion that behavioral extinction during reconsolidation can lead to persistent attenuation of conditioned fear.

Evidence from rodents has shown that the neural mechanism of action for the reconsolidation of fear memories is a cascade of molecular events taking place in the lateral nucleus of the amygdala (Monfils et al., 2009; Nader et al., 2000; Duvarci and Nader, 2004; Debiec and LeDoux, 2004). Recent human imaging studies have reiterated this finding by demonstrating behavioral interference during reconsolidation led to persistently attenuated fear at a subsequent recovery test, independent of PFC involvement (Agren et al., 2012), as well as showing that if fear extinction occurred during reconsolidation, decreases in fear response were not mediated by prefrontal activity (Schiller et al., 2013). These findings support the notion that reconsolidation update is a pre-frontally independent method of fear regulation. This makes it a potentially attractive alternative to extinction for adolescents, a developmental group characterized by protracted development of prefrontal regions upon which successful extinction is dependent. It may also be helpful for individuals with genetic profiles that cause them to respond poorly to exposure-based therapies that presumably rely on prefrontally-mediated regulation of fear. We tested this method in adolescent humans and the results of this experiment are discussed in Chapter 4 (Johnson & Casey, 2015b).

The current thesis

The goal of this thesis is to characterize developmental and individual differences in fear extinction learning in humans and to test methods to enhance fear regulation in these populations. The format for the current thesis is as follows: Chapter 1, "Introduction", provides background information necessary to set the stage for eludicating the role of fear extinction learning in the etiology and treatment of stress and

anxiety disorders. I lay the groundwork for an exploration of the roles that developmental stage and genetic profile play in mediating these regulatory processes, as well as introduce possible alternatives to extinction learning that could lead to enhanced fear regulation[1]. Chapter 2, "Diminished fear extinction learning in adolescents", (Pattwell, Duhoux, Hartley, Johnson, et al., PNAS, 109(40), 16318-16323) provides evidence from humans that the developmental stage of adolescence is a time during which fear extinction learning is diminished. Chapter 3, "Parallel effects of FAHH in fear regulation", (Divencha, Drysdale, Hartley, Johnson[2], et al., 2015) demonstrates that variation in the FAAH gene mediates the rate of fear extinction learning in human adults. Chapter 4, "Fear regulation during adolescence: a matter of timing" (Johnson & Casey, 2015b), demonstrates that a method based on the principles of memory reconsolidation, reconsolidation update, can be utilized to block the recovery of fear in adolescent humans. Chapter 5, "Conclusions", synthesizes the findings presented in Chapters 2 through 4, as well as discussing limitations, clinical implications and future directions.

[1] Parts of this chapter originally appeared in Johnson & Casey (2015a), Easy to remember, difficult to forget: The development of fear regulation. *Developmental Cognitive Neuroscience*, *11*, 42-55

[2] Co first author

Chapter 2

Altered fear learning across development in humans

Introduction

Fear learning is a highly adaptive, evolutionarily conserved process that allows one to respond appropriately to cues associated with danger. In the case of psychiatric disorders, however, fear may persist long after an environmental threat has passed. This unremitting and often debilitating form of fear is a core component of many anxiety disorders, including posttraumatic stress disorder (PTSD), and involves exaggerated and inappropriate fear responses. Existing treatments, such as exposure therapy, are based on principles of fear extinction, during which cues previously associated with threat are presented in the absence of the initial aversive event until cues are considered safe and fear responses are reduced. Extinction-based exposure therapies have the strongest empirical evidence for benefitting adult patients suffering from PTSD (Rothbaum et al., 2003), yet a comparative lack of knowledge about the development of fear neural circuitry prohibits similarly successful treatment outcomes in children and adolescents (Liberman et al., 2006). Adolescence, in particular, is a developmental stage when the incidence of anxiety disorders peaks in humans (Monk et al., 2003; Kessler et al., 2005; Merikangas et al., 2011; Newman et al., 1996), and it is estimated that over 75% of adults with fear-related disorders met diagnostic criteria as children and adolescents (Pollack et al., 1996; Kim-Cohen et al., 2003). Because of insufficient or inaccurate diagnoses and a dearth of pediatric and adolescent specialized treatments, fewer than one in five children or adolescents are expected to receive treatment for their anxiety disorders (Merikangas

et al., 2010), leaving a vast number with inadequate or no treatment (Liberman et al., 2006; Keller et al., 1992). The increased frequency of anxiety disorders in pediatric and adolescent populations highlights the importance of understanding neural mechanisms of fear regulation from a developmental perspective, as existing therapies directly rely upon principles of fear-extinction learning. Converging evidence from human and rodent studies suggests that insufficient top-down regulation of subcortical structures (Levesque et al., 2004; Galvan et al., 2006; Hare et al., 2008; Casey et al., 2010), such as the amygdala, may coincide with diminished prototypical extinction learning (Milad & Quirk, 2012), as well as ongoing fine-tuning of excitatory–inhibitory balance in the prefrontal cortex that may coincide with diminished prototypical extinction learning (Hensch et al., 2005). Because top-down prefrontal regulation has been postulated to mediate extinction learning and may determine the efficacy of exposure therapy often used as part of cognitive behavioral therapy, it is important to discern how changes in the development of prefrontal circuitry influences fear extinction. Studying the development of fear learning and memory in humans, while examining, in parallel, the underlying neural mechanisms in rodent models, may offer insights into optimizing treatment strategies for developing populations by clarifying when, during development, a particular intervention or treatment may be more or less effective.

Methods

Participants

Eighty-three healthy volunteers, including 30 children (5–11 y old; 14 male, mean age = 8.8 y), 28 adolescents (12–17 y old; 15 male; mean age = 13.9 y), and 25 adults

(18–28 y old; 12 male, mean age = 22.8 y), completed the study. An additional 42 participants (13 children, 16 adolescents, 13 adults), were excluded from analysis because they either didn't show measurable skin conductance response (SCR) (n = 20, 5 children, 10 adolescents, 5 adults), failed to differentiate between the threat and safety cues [i.e., no difference in mean galvanic skin response to conditioned stimulus (CS)+ compared with CS− in any run of the acquisition session or in the first run of the extinction session] (n = 17, three children, six adolescents, eight adults) or requested to stop participating before the conclusion of the study (five children). In addition, the four youngest females in the adult cohort were excluded to balance the sex ratios within each group. Trait anxiety was assessed using the Spielberg State-Trait Anxiety Inventory trait subscale (STAI-T). Trait anxiety ratings were collected from all but three participants (one child, one adolescent, and one adult). Pubertal development was assessed through self-report or parent reports for two standardized scales (Petersen et al., 1988; Tanner, 1962), resulting in an averaged Tanner staging correspondence of Tanner stage 1 to preadolescence, Tanner stages 2, 3, and 4 to adolescence, and Tanner stage 5 to postadolescence.

Experimental Design

Participants underwent a partial-reinforcement discriminative fear-conditioning paradigm spanning 2 days (Figure 2.1A). Two colored squares were used as stimuli. On day 1, one square (CS+) was paired on 50% of the trials with an aversive sound [unconditioned stimulus (US)]. The other square was never paired with the aversive sound (CS−). The color of the CS+ and CS− was counterbalanced across subjects. The partial pairing of the US with the CS+ allowed us to analyze the response to the CS+

independently of the response triggered by the tone. One day later, all participants underwent extinction training wherein the CS+ and CS− were presented repeatedly without the US. The paradigm was adapted from one previously used (Soliman et al., 2010). Participants were not informed about the goals of the study; they were told to attend to the stimuli, to press a key when each square appeared on the screen (to ensure attention), and that they may occasionally hear a loud noise. Each CS was presented for 3 s each, with a 13-s intertrial interval (ITI). The duration of the ITI was set to ensure that SCR responses could return to baseline and allow responses to be decoupled between trials. On reinforced CS+US trials, the CS+ coterminated with the US. The conditioning session consisted of 12 CS+US, 12 nonreinforced CS+, and 24 CS− trials. Extinction consisted of 24 CS− and 24 nonreinforced CS+ trials. Stimuli were presented in pseudorandomized order, avoiding consecutive CS+USs during the conditioning session and allowing no more than two consecutives squares of the same color in either session. Both the conditioning and extinction sessions were divided into three runs of 16 trials each, with each run lasting 4 min 26 s; a brief break occurred between each run. The auditory stimulus was a white noise combined with a 1,000-Hz tone, which was intensity tiered for smooth onset and offset. It was rated as aversive in an independent experiment (Levita et al., 2009). The sound was delivered in both ears, through headphones. Sound intensity was tested before each experiment using an audiometer, and subjects chose a sound level that was very unpleasant but not painful. The sound intensity level ranged from 85 dB to 95 dB (mean ± SD; 92.5 ± 2.9) in children, from 94.5 dB to 107 dB in adolescents (96.5 ± 2.4), and from 94 dB to 104 dB in adults (96.5 ± 2.4). The duration of the auditory stimulus was 1 second.

Physiological Measurement and Analysis

Skin conductance response (SCR) was acquired using disposable snap electrodes pre-gelled with isotonic gel, which were attached to the distal phalanx of the second and third digits of the left hand. The signal was recorded and amplified using a skin conductance recording system (MP35; Biopac) in combination with AcqKnowledge software (Biopac). E-prime software (Psychology Software Tools) was used to control the presentation of visual and auditory stimuli and send time markers to the skin conductance recording system for each context and stimulus onset/offset. The SCR was sampled at 200 Hz with a 1-HZ filter applied. SCR was analyzed manually. For each individual subject, data were smoothed. Measurable peaks were identified as the first SCR response that occurred within .5-4.5 s following stimulus onset as defined by the difference between trough and peak being equal to or greater than .02 uS (microsiemens)(Schiller et al., 2013). A zero value was added into the analysis when no peak was detected. SCR scores were square root-transformed to normalize the distribution and were then scaled to the participant's largest response to the CS+US during acquisition to normalize responses across participants. These SCR scores were averaged for each participant for each stimulus type separately. All SCR responses reported for acquisition reflect differences between responses to the CS+ and corresponding CS-. SCR responses reported for extinction reflect responses to the CS+ only.

Results

Immature functional connectivity between the ventromedial prefrontal cortex (vmPFC) and amygdala in adolescents has previously been shown in tasks of emotion regulation (Hare et al., 2008). Therefore, we sought to investigate age-dependent differences in fear-extinction learning in humans, as cumulative evidence suggests a relationship between fear extinction learning capacity and functional connectivity between these same neural regions (Milad and Quirk, 2012). Using age delineations for children, adolescents, and adults, we assessed skin conductance response across development in humans to measure prototypical physiological fear responses during conditioned fear acquisition and fear memory extinction (Pattwell et al., 2011; Soliman et al., 2010; Laviola et al., 1999; Adriani et al., 1998; LeDoux 2000).

A two-way ANOVA on skin conductance response during late acquisition (the last of three acquisition runs) with main factors of age group (children, adolescents, adults) and stimulus type [paired conditioned stimulus (CS+) or unpaired (CS−) with an aversive noise] showed a main effect of stimulus type (CS+ > CS−) [$F(1, 79) = 10.786$, $P = 0.002$] and no Group x Stimulus type interaction [$F(2, 79) = 0.032$, $P = 0.968$], demonstrating that all subjects learned to discriminate between the threat cue and the safety cue (Figure 2.1).

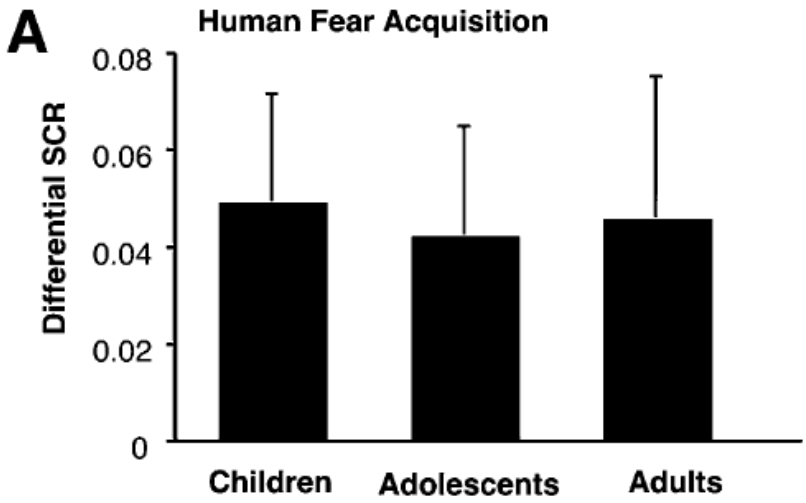

Figure 2.1. Cued fear acquisition across development in humans. (A) There were no developmental differences in fear acquisition in the human subjects.

Furthermore, there was no main effect of age group on responses to either stimulus type [CS+: $F(2,79) = 0.581$, $P = 0.562$; CS−: $F(2, 79) = 0.655$, $P = 0.522$] or the differential acquisition measure [CS+ − CS−: $F(2, 79) = 0.021$, $P = 0.979$] during late acquisition. Thus, any subsequent group effects in extinction learning are not related to differences in fear acquisition. In contrast, analysis of extinction indices revealed a main effect of age group for humans [$F(2, 80) = 3.228$, $P = 0.038$], such that adolescents showed attenuated fear-extinction learning compared with children [$t(56) = 2.34$, $P = 0.023$] and a trend towards attenuated fear-extinction learning compared to adults [$t(51) = 1.802$, $P = 0.078$] (Fig. 2.2B). This effect of age group on fear extinction was present when sex and trait anxiety are entered as covariates [$F(2, 73) = 3.086$, $P = 0.052$]. There was no significant difference in extinction learning between children and adults ($P = 0.701$).

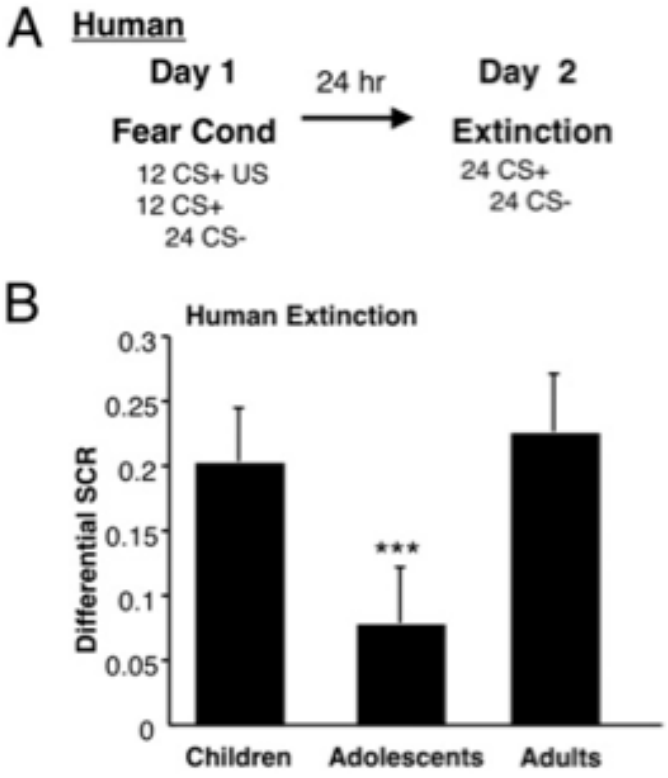

Figure 2.2. Cued extinction learning and spontaneous recovery across development in humans. (A) Behavioral paradigms for fear conditioning experiments in humans. (B) Analysis of extinction indices [(averaged first two extinction trials) − (averaged last two extinction trials)] reveals a main effect of age group for humans, such that adolescents display attenuated fear extinction learning compared with children and adults, (adolescent 0.05916 ± 0.06904; children 0.25435 ± 0.04839; adults 0. 22510 ± 0.05931)

Discussion

Adolescence is a highly conserved developmental stage, both neurobehaviorally and physiologically, during which all mammals must meet evolutionary pressures associated with sexual emergence and transition from dependence on parents to independence (Spear 2004). Fear learning plays a critical adaptive role in this process as the adolescent leaves the relatively protected and stable family environment and explores a novel and highly variable outside environment. Defining the unique attributes of fear

acquisition and extinction during adolescence may have wide clinical implications, as the most common and validated treatment for anxiety disorders involves exposure-based therapy, which relies heavily on extinction principles for reevaluating existing contingencies (Rothbaum & Davis, 2003).

Parallel studies in the rodent were conducted simultaneous to the human study presented here. Conservation of the behavioral demands associated with adolescence provides face and construct validity for translational studies of fear learning in this age group. The animal studies presented below in support of the human studies are particularly important because human experiments are limited in the extent to which the underlying molecular mechanisms can be probed. Fear learning processes are highly conserved across species and evolutionary time. This suggests that diminished fear learning might be observed in adolescents across species and that a neural mechanism common to both humans and rodents may underlie this age-specific behavioral phenotype. Pattwell et al (2012a) conducted parallel Pavlovian fear-learning experiments in preadolescent (P23), adolescent (P29), and adult (P70) mice to assess developmental differences in conditioned fear acquisition and extinction learning at ages comparable to human children, adolescents and adults (Spear 2000; McCallum et al., 2010) (Fig 2.2A). In these experiments, Pattwell et al. (2012) showed diminished extinction learning and retention of extinction memory in adolescent mice (P29) compared to younger (P23) and older mice (P70), as indexed by freezing, a pattern of results similar to that observed in the humans (Fig 2.1B).

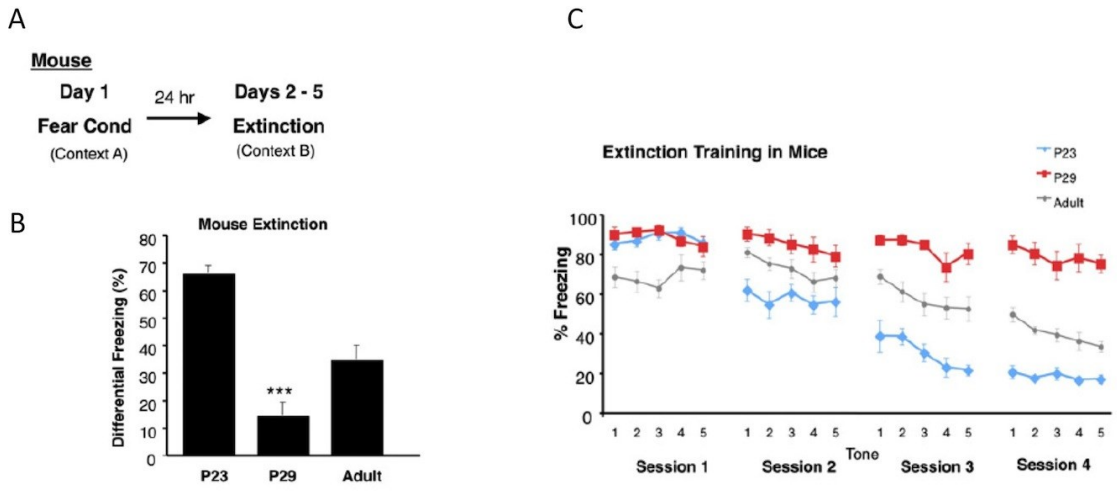

Fig. 2.3. Cued extinction learning across development in mice. (A) Behavioral paradigms for fear conditioning experiments in mice. (B) A lack of extinction learning and retention of extinction memory in is observed in adolescent mice, as displayed by a significantly decreased differential extinction indices [(Day 1, Tone 1) − (Day 4, Tone 5)] compared with older and younger ages, (P23 66.5% ± 2.75; P29 14.72% ± 4.79; P70 35.17% ± 4.89). (C). Adolescent mice display attenuated extinction learning over the course of four days compared with preadolescent and older adult mice. From Pattwell et al., 2012a.

Immunohistochemical and electrophysiological experiments showed that the mechanism underlying this age-specific difference in extinction learning is immature connectivity between infralimbic cortex and the amygdala (Pattwell et al., 2012a). Strong cross-species preservation of the neural circuitry implicated in fear-extinction learning is supported by human and nonhuman animal extinction studies, further bolstering the translational credibility of rodent experiments to explore mechanistic underpinnings that are otherwise precluded from experiments with human subjects (Soliman et al., 2010; Gottfried & Dolan 2004).

Collectively, the human data presented in this chapter and mouse data from the Pattwell et al (2012a) studies have demonstrated attenuated extinction learning, at a

defined developmental stage, in both mice and humans. Through performing parallel human and mouse studies examining fear acquisition and extinction, it was possible to uncover similar developmental patterns in fear-extinction learning, lending credibility to the use of a developmental mouse-model system for examining human adolescent fear and anxiety. Confirming that similar developmental patterns in fear-extinction behavior exist for both mice and humans allows for the use of the mouse model system to probe underlying physiological mechanisms responsible for the attenuated fear-extinction learning observed in adolescence. Earlier studies have shown changes in intrinsic properties of the vmPFC neurons after fear acquisition and extinction (Burgos-Robles et al., 2009; Santini et al., 2008; Burgos-Robles et al., 2008). However, the specific involvement of the vmPFC excitatory synapses in fear learning or extinction was unclear. Findings from the Pattwell et al. (2012a) mouse studies showing potentiation of the PL excitatory synapses after fear acquisition in P23 and adult mice, and their subsequent depotentiation upon extinction, suggest that the PL excitatory synapses dynamically regulate fear expression. More importantly, the simultaneous potentiation of the IL excitatory synapses in adult fear-extinguished mice provides an additional mechanism by which vmPFC excitatory synapses mediate extinction. The PL projects to the basolateral amygdala and might exert excitatory effects on the central amygdala to enhance fear (Vertes, 2004; Vertes, 2006), but on depotentiation, the PL L5 pyramidal neurons might reverse this fear-enhancing effect. In addition, the enhanced glutamatergic IL output during fear extinction might facilitate the intercalated cell-mediated feed-forward inhibition of the central amygdala, resulting in decreased fear response (Pare et al., 2004; Milad & Quirk, 2002; Quirk et al., 2006; Likhtik et al., 2005; Phelps et al., 2004).

However, these synaptic plasticity changes in the PL and IL observed in P23 and adult mice are absent in adolescent mice, suggesting that the vmPFC is not similarly engaged in the regulation of learned fear at this age. Given the delayed development of cortical GABAergic transmission, it is plausible that an imbalance in inhibitory synaptic transmission during adolescence interferes with synaptic plasticity in the mPFC (Chattopadhyaya et al., 2004; Kilb 2011). These experiments identify unique synaptic properties in the vmPFC that may underlie developmentally regulated differences in fear extinction that extend to the human species.

During adolescence, there is altered vmPFC synaptic activity and decreased fear-extinction behavior compared with younger and older ages, which may provide insights into the efficacy of treatments for anxiety disorders that rely on extinction mechanisms during this developmental period. In particular, the human data presented here, along with the supporting mouse data, suggest that treatment response to exposure-based cognitive behavioral therapy would vary nonlinearly across age, with the poorest response in adolescents, highlighting the importance of optimizing treatment strategies based on age.

Chapter 3

Parallel effects of genetic variation in endocannabinoid signaling on frontolimbic circuitry and function in adult humans

Introduction

The endocannabinoid system has been implicated in human anxiety (Hariri et al., 2009; Gunduz-Cinar et al., 2013a; Hill et al., 2008). The ligand, anandamide (AEA), an endogenous cannabinoid (eCB) that binds to the CB_1 receptor, is proposed to play a central role in this effect (Gunduz-Cinar et al., 2013a; Gunduz-Cinar et al., 2013b). FAAH is a catabolic enzyme and primary regulator of AEA signaling in the brain (Cravatt et al., 2005). In humans, differential expression of FAAH protein is associated with a common SNP (C385A; rs324420) for which ~38% of individuals of European descent are carriers (AC, AA genotypes) (Abecasis et al., 2012). This polymorphism leads to the substitution of an evolutionarily conserved proline at amino acid position 129 with a threonine residue, which in turn renders the FAAH protein more vulnerable to proteolytic degradation. Accordingly, the FAAH C385A SNP enhances eCB signaling by reducing steady state levels of FAAH protein, which leads to elevated AEA levels (Sipe et al., 2002; Sipe et al., 2010; Chiang et al., 2004). In humans, FAAH C385A has been associated with variation in reactivity to threat (Hariri et al., 2009). While animal studies featuring pharmacologic manipulations and genetic knockout of FAAH have shown FAAH C385A is implicated in anxiolytic behaviors including enhanced fear extinction learning (Gunduz-Cinar et al., 2013a; Kathuria et al., 2003; Chhatwal et al., 2005), the effect of the FAAH C385A SNP to facilitate enhanced fear extinction learning has not

previously been tested in humans. In this study, a Pavlovian fear conditioning paradigm was used to test the effect of the FAAH C385A SNP on fear extinction learning in human adults. Additionally, the anxiolytic phenotype of the FAAH variant A allele was characterized using standard measures of anxiety (STAI; State-Trait Anxiety Inventory).

Methods

Participants

40 adult volunteers completed the study (Non A carriers: n = 22; 13f, Mean age = 25.2, sd= 2.8; 9m, mean age = 24.4, sd = 4.7; A carriers: n = 18, 8f, mean age = 20.25, sd = 4.7, 10m, mean age = 23, sd = 4.7). All participants were screened for exclusionary criteria, including hearing impairment, color blindness, diagnosed animal phobias and neurological and psychiatric disorders, as well as provided written consent prior to the experiment. Trait anxiety was assessed using the Spielberg State-Trait Anxiety Inventory trait subscale (STAI-T).

Experimental Procedure

We utilized a two-day fear conditioning/extinction paradigm. Acquisition occurred on experimental day 1 and extinction occurred 24 hours later on experimental day 2. Acquisition and extinction took place in different visual contexts consisting of pictures of rooms presented on the computer screen (Context A & B) (Milad et al., 2005). Cues were two colored windows (blue and yellow) that were otherwise black, embedded within each visual context. The unconditioned stimulus (US) was a hybrid consisting of auditory and visual components. The visual component was derived from a set of

validated aversive pictures from the International Affective Picture Series (Lang et al., 2005). The auditory component was a validated custom-designed hybrid of white noise and a 1000Hz tone with duration of one second, intensity tiered for smooth onset and offset (Pattwell et al., 2012; Soliman et al., 2010; Levita et al., 2009). Fear acquisition took place in Context A. One cue was paired with the US at a 50% reinforcement rate (CS+). Each presentation of the US consisted of the same sound and a different picture. The other cue (CS-) was never followed by the US. Participants were presented 32 trials during acquisition (8 CS+US, 8 CS+ and 16 CS-). Twenty-four hours later, participants returned for experimental day 2, which took place in Context B and consisted of a 32-trial extinction session (16 CS+, 16 CS-). Stimuli were presented in a pseudo-randomized order, defined by non-consecutive CS+USs during conditioning and no more than three consecutive squares of the same color in any session. Visual contexts, stimuli and script orders were counterbalanced across subjects. Fear response was measured by skin conductance response (SCR), an index of autonomic nervous system activity (LaBar et al, 1998). Differential fear responding was calculated by subtracting normalized and scaled SCR to the CS- from corresponding CS+ responses. Only subjects who showed fear acquisition (magnitude of SCR to the CS+ was greater than to the CS- during acquisition) were included in the analyses. In this experiment, half the participants were randomly assigned to either a reconsolidation update or extinction only condition. Participants who were assigned to the reconsolidation update condition received a single presentation of the CS+, unpaired with the aversive stimulus, prior to a 10-minute rest period followed immediately by the extinction session.

Physiological Response Measurement and Analysis

Skin conductance response (SCR) was acquired using disposable snap electrodes pre-gelled with isotonic gel, which were attached to the distal phalanx of the second and third digits of the left hand. The signal was recorded and amplified using a skin conductance recording system (MP35; Biopac) in combination with AcqKnowledge software (Biopac). E-prime software (Psychology Software Tools) was used to control the presentation of visual and auditory stimuli and send time markers to the skin conductance recording system for each context and stimulus onset/offset. The SCR was sampled at 200 Hz with a 1-HZ filter applied. SCR was analyzed manually. For each individual subject, data were smoothed. Measurable peaks were identified as the first SCR response that occurred within .5-4.5 s following stimulus onset as defined by the difference between trough and peak being equal to or greater than .02 uS (microsiemens)(Schiller et al., 2013). A zero value was added into the analysis when no peak was detected. SCR scores were square root-transformed to normalize the distribution and were then scaled to the participant's largest response to the CS+US during acquisition to normalize responses across participants. These SCR scores were averaged for each participant for each stimulus type separately. All SCR responses reported reflect differences between responses to the CS+ and corresponding CS-.

SCR was recorded at a sampling frequency of 200Hz with a 1Hz filter and manually smoothed. After each stimulus presentation, the first SCR peak occurring within 0.5-4.5s was considered the subject's response to that stimulus. Responses, as estimated by the difference between trough and peak, were only included in analysis

when greater than 0.02μS (Schiller et al., 2010a). Responses smaller than this threshold were replaced with zeros during analysis. During analysis, individual SCR responses were square root transformed and scaled to that participant's largest CS+US acquisition response. Normalized SCR scores were averaged separately by stimulus type (CS+, CS-, CS+US). All presented responses were calculated as the difference between CS+ and CS- responses. We binned extinction trials into early (mean differential CS+ responses of trials 1 through 5) and late blocks (mean differential CS+ responses during trials 6 through 11). Only subjects who showed fear acquisition (magnitude of SCR to the CS+ was greater than to the CS- during acquisition) were included in the analyses.

Results

We tested humans (22 CC-alleles, 18 A-allele carriers; Table 3.1), using cued fear conditioning with extinction on the following day. There was no main effect of FAAH genotype on fear acquisition [t(38) = -1.013] (Figure 3.1), suggesting that any subsequent group differences in fear extinction are not related to strength of conditioning.

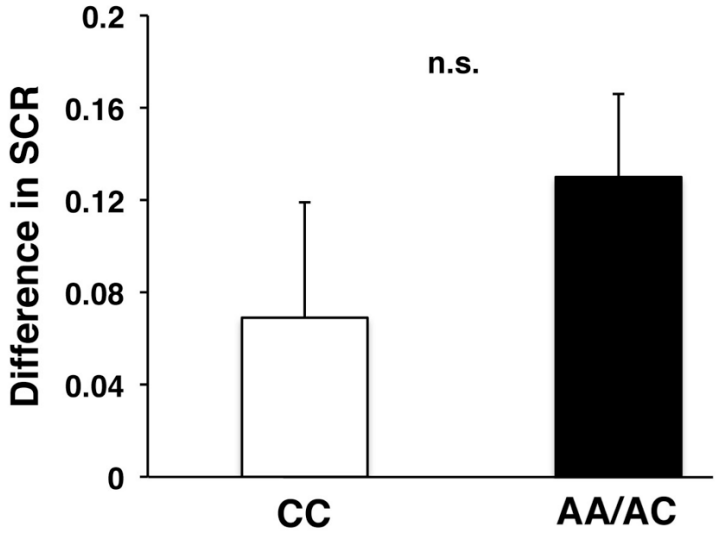

Figure 3.1. Fear acquisition in humans with FAAH C385A polymorphism. Fear acquisition in A allele carriers (n = 18) relative to C homozygotes (n = 22) as measured by calculating differential skin conductance responses (SCR) between a cue previously paired with an aversive stimulus (CS+) and a cue never paired with an aversive stimulus (CS-) on the last 3 trials of acquisition [t(38) = -1.013]

Human A allele carriers showed facilitated fear extinction learning, as indexed by decreased galvanic skin responses during late trials of extinction training [($F(1,36)=4.35$, $P < 0.044$)], controlling for potential age and gender effects between genotypic groups (Fig. 3.2a). This is in accordance with parallel findings in mice (Divencha et al., 2014) that showed a significant effect of genotype on freezing behavior during extinction (ANOVA with post-hoc Bonferroni test [$F(2,116)=6.8$, $P < 0.01$]) (Fig. 3.2b), with heterozygous [$P < 0.05$] and homozygous [$P < 0.01$] FAAH C385A mice showing less freezing behavior compared to wild types in late trials, but no difference in early trials (Fig. 3.2b) or in fear acquisition. These parallel effects of enhanced fear extinction in FAAH A-allele carriers have not previously been established for both mice and humans.

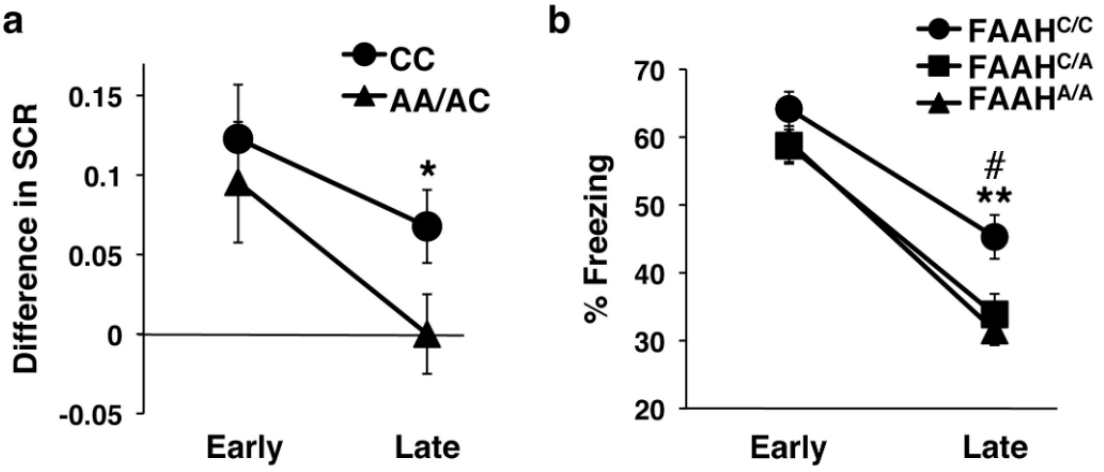

Figure 3.2. Enhanced Cued Fear Extinction in humans and mice with FAAH C385A. (a) Extinction, indexed by differential skin conductance response (SCR) [(CS+) – (CS-)], in 18 A allele carriers and 22 C homozygotes. Trials were binned into early (average of the first 5 trials) and late (average of the following 6 trials). (**b**) Fear extinction, time spent freezing to cue, was tested in wild type ($FAAH^{C/C}$; n = 21), heterozygous ($FAAH^{C/A}$; n = 20) and homozygous knock-in mice ($FAAH^{A/A}$; n = 20). Extinction trials were binned into early (average of extinction day 1) and late trials (average of extinction day 4). Means ± SEM presented. *$P < 0.05$, $^{\#}P < 0.05$ heterozygous knock-in mice vs wild-type controls, **$P < 0.01$ homozygous knock-in mice vs wild-type controls.

We further characterized the anxiolytic phenotype of the FAAH variant A allele in our human participants using standard measures of anxiety-related behaviors (STAI; State- Trait Anxiety Inventory). In accordance with our results showing enhanced fear extinction in human and mouse FAAH A-allele carriers, reduced levels of trait anxiety were reported by A allele carriers [t(38) = -2.24, p = .03](Figure 3.3A), an effect also seen in a separate cohort of 137 humans [t(135)=2.30, *P* = 0.019] (Figure 3.3B) (Divencha et al, 2014).

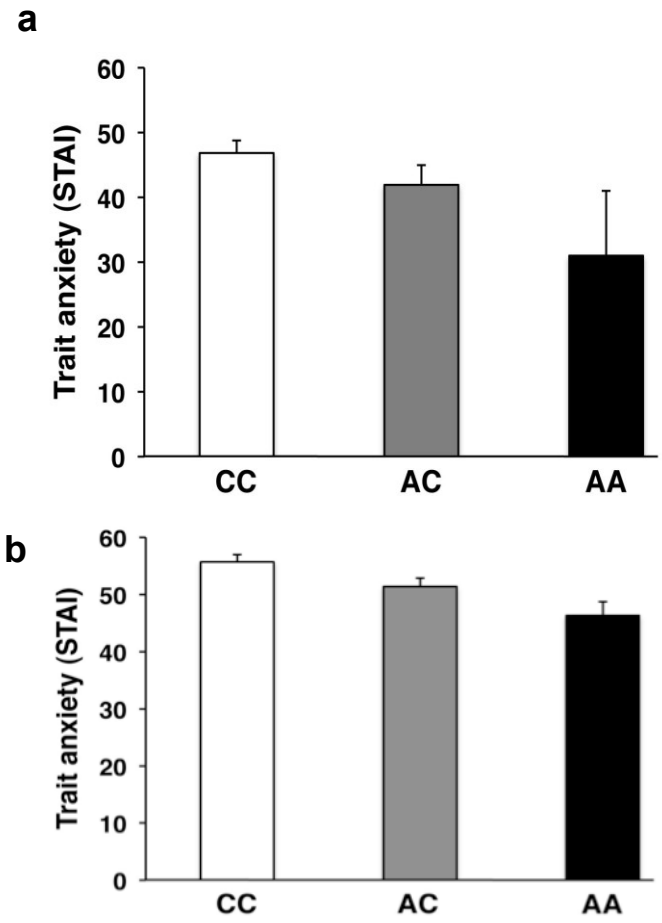

Figure 3.3. Inverse relationship between FAAH C385A and trait anxiety in two distinct human adult populations. (a) Relationship between number of mutant FAAH C385A alleles and STAI trait anxiety was examined in the human adults in the present study, revealing a significant negative correlation [t(38)=-2.247, *P* = 0.03]. **(b)** A separate population of 137 adults showed a similar negative correlation [t(135)=2.30, *P* = 0.019] (Divencha et al., 2015). Means ±SEM presented.

Discussion

The data presented here provides evidence that the FAAH 385A SNP leads to enhanced fear extinction learning and decreased trait anxiety in human adults. While these results are derived from a small population relative to that typically seen in genetics studies, these findings are supported by concordant data from a set of parallel human and animal studies. The animal studies assessed fear extinction learning and trait anxiety utilizing a knock-in mouse that expresses the variant A (threonine) allele of the FAAH polymorphism in place of the conserved ancestral C (proline) allele, recapitulating the genotypic variation naturally observed only in humans. These studies replicated the behavioral findings from the human extinction sample and provided the opportunity to characterize the effects of the FAAH variant in the brain. The human studies examined the effect of the FAAH 385A SNP on the neural circuitry underlying fear acquisition and extinction learning through analysis of resting state fMRI (rs-fMRI) data, as well as replicating the trait anxiety findings in a separate population of 137 adult subjects. These studies enabled the demonstration of parallel molecular, circuit-level and behavioral phenotypes in humans and in the knock-in mice carrying this variant **(**Divencha et al., 2015**)** and is presented below.

Enhanced connectivity between ventromedial prefrontal cortex (vmPFC) and amygdala in humans and mice with the FAAH C385A polymorphism

In humans, FAAH C385A has been associated with variation in reactivity to threat (Hariri et al., 2009). However, it is unclear how the FAAH C385A polymorphism might alter the circuitry implicated in this behavioral domain. Fear conditioning studies

in animal models suggest that dynamic interaction between the amygdala and two subregions of the prefrontal cortex (PFC) can drive opposing behavioral responses to threat (Vidal-Gonzalen et al., 2006; Laurent & Westbrook, 2009; Sierra-Mercado et al., 2011; Phelps & LeDoux, 2005). Whereas the prelimbic region (PL) promotes fear expression, the infralimbic region (IL) promotes the regulation of threat responses. Neuroimaging studies examining correlates of these opposing behaviors in humans suggest that a dorsal anterior cingulate cortex (dACC) region exhibits functional parallels to rodent PL and a subgenual region of ventromedial PFC (vmPFC) exhibits functional parallels to rodent IL (Hartley & Phelps, 2010; Phelps et al., 2004; Milad et al., 2007; Schiller & Delgado, 2010b).

Species-specific connectivity analyses were performed focusing on key regions within the frontoamygdala circuitry that regulate fear responses and fear extinction learning. First, resting state connectivity between the subgenual vmPFC and the amygdala and the dorsal ACC and the amygdala was examined in humans by genotype. A allele carriers showed increased correlation between the blood oxygen level-dependent (BOLD) signals in the vmPFC and the bilateral amygdala (Divencha et al., 2014). This pattern was selective to subgenual vmPFC–amygdala connectivity with no genotypic difference in dorsal ACC-amygdala connectivity. A substantial literature suggests that stronger inverse functional connectivity between the vmPFC and the amygdala during emotion-related tasks is associated with decreased anxiety or negative emotion (Kim et al., 2004; Urry et al., 2006; Hare et al., 2008). In contrast, positive resting state functional connectivity between these regions has been associated with lower anxiety (Kim et al., 2011; Burghy et al., 2012; Hahn et al., 2011). The human resting state analysis is

consistent with this broader literature suggesting that greater positive corticoamygdala connectivity at rest predicts more effective emotional control (Kim et al., 2011a; Kim et al., 2011b).

Anterograde (adeno-associated virus expressing enhanced green fluorescent protein; AAV2-eGFP) and retrograde (fluorogold) tracers were used in the FAAH knock-in mouse to delineate the precise location and directionality of genotypic differences in frontoamygdala circuitry. These tract-tracing experiments revealed increased IL to basolateral amygdala (BLA) projections in mice, but no genotypic differences in ascending projections from the BLA to the IL. Mirroring the human imaging findings, no genotypic differences in descending or ascending projections were found between the PL and BLA in the mouse (Divencha et al., 2014). Selective increases in descending IL-amygdala projections offer a structural neuroanatomical basis for the increased functional connectivity in frontoamygdala circuitry in human A allele carriers and may help explain reported genotypic differences in emotion regulation (Hariri et al., 2009).

Enhanced cued fear extinction in mice with the FAAH C385A polymorphism

Mice showed a significant effect of genotype on freezing behavior during extinction with heterozygous and homozygous FAAH C385A mice showing less freezing behavior compared to wild types in late trials, but no difference in early trials or in fear acquisition (Figure 3.2b) (Divencha et al., 2014). These results are consistent with reported effects of pharmacological and genetic knockout manipulations of FAAH expression in mice (Gunduz-Cindar et al., 2013; Moreira et al., 2008; Chhatwal & Ressler, 2007) and with the effects reported here in human adults. These parallel effects

of enhanced fear extinction in FAAH A-allele carriers have not previously been established for both mice and humans.

Decreased anxiety levels in mice and humans with the FAAH C385A polymorphism

In accordance with the data reported here, the anxiolytic phenotype of the FAAH variant A allele was characterized in both mice and an additional cohort of humans using standard measures of anxiety-related behaviors. In mice, two standard measures of anxiety-like behaviors were performed that place subjects in conflict situations. In an elevated plus maze (EPM) test, homozygous mutant mice spent a higher percentage of time in the open arms than wild-type controls, indicative of reduced anxiety-like behavior. Mice in the novelty-induced hypophagia (NIH) task were trained to approach a reward (sweetened milk) in their home cage and then tested in a novel, brightly lit cage. Within this task, the latency to approach and drink the sweetened milk is a measure of anxiety-related behavior (Dulawa & Hen, 2005). FAAH C385A mice showed decreased latency to drink milk in the NIH task, suggesting decreased anxiety phenotype. Neural correlates of the observed anxiolytic effects were explored by examining neural activity, as indicated by expression of the early immediate gene c-fos (Dragunow and Faull, 1989), in the BLA following the NIH task. Data showed a main effect of genotype on BLA c-Fos expression in mutant knock-in mice in a dose-dependent pattern, suggesting decreased engagement of the BLA in these mice during a stressful exposure (Divencha et al., 2014). This reduced activation of the amygdala in response to stressful stimuli is consistent with reductions in amygdala activity in response to threat in human FAAH A

allele carriers (Hariri et al., 2009). In an additional cohort of 137 humans, A allele carriers reported reduced levels of trait anxiety (STAI; State-Trait Anxiety Inventory), replicating the pattern shown in the human fear extinction sample (Figure 3.3b) (Divencha et al., 2014). Collectively, this evidence from mice and humans suggests the FAAH 385A polymorphism is associated with lower trait anxiety.

Using a vertically integrated approach in parallel studies in humans and mice, these studies collectively provide converging evidence supporting the relevance and impact of the FAAH C385A polymorphism on brain biochemistry, neurocircuitry, behavior and symptoms. The mouse model was validated by showing that the FAAH variant allele leads to reductions in FAAH protein and enzymatic activity and increases in AEA levels in the brain. Subsequent analyses in humans and mice elucidated circuit level and behavioral phenotypes not previously established in human carriers with this SNP. Enhanced fear extinction has been shown in both humans and mice with the C385A mutation. In addition, the finding of selectively enhanced frontoamygdala connectivity in both mouse and human carriers provides a mechanistic explanation for these behavioral effects of the A allele through enhanced regulation of basolateral amygdala responses to threat by the infralimbic (mouse) and ventral medial prefrontal (human) cortex. Thus, this variant SNP may represent a gain-of-function in the domain of anxiety-related behaviors and may prove valuable in determining for whom and for what symptoms FAAH inhibitors or exposure-based therapies that build on basic principles of extinction learning will be most efficacious. In this way, therapeutics might be tailored to an individual to move from standard care to more precise personalized care. A persistent problem identified in animal models of disease is a failure of the findings to translate to the human

in clinical trials. These studies translate mouse behavioral and brain findings to show their human relevance using parallel paradigms and imaging tools across species. Thus, this work and integrated approach fill a large translational gap from mouse to human.

Chapter 4

Extinction during memory reconsolidation blocks recovery of fear in adolescents

Introduction

Fear is an adaptive function that allows an individual to respond appropriately to the imminent arrival of danger. For most individuals who experience such events, fear responses naturally extinguish across time as the danger diminishes. However, when the fear persists long after the danger has passed, this can lead to the development of stress and anxiety-related disorders. These disorders are the most common of all the psychiatric illnesses and frequently emerge during adolescence, often persisting into adulthood (Merikangas et al., 2010), with a majority of all adults first meeting diagnostic criteria during childhood or adolescence (Kim-Cohen et al., 2003).

The most effective evidence-based behavioral treatment for anxiety and stress-related disorders is exposure-based cognitive behavioral therapy (CBT) (Rothbaum et al., 2010). Exposure-based CBT is based on the principles of fear extinction and involves identifying the triggers for the underlying anxiety and desensitizing the patient to these triggers with repeated exposures. As presented in Chapter 2, emerging evidence from both rodents and humans suggests that adolescents are less capable of extinguishing fear memories relative to younger or older animals (Pattwell et al., 2012a; McCallum et al., 2010). While the amygdala, medial prefrontal cortex and hippocampus are known to constitute a core neural circuit in fear extinction learning (LeDoux 2000), this circuit is functionally immature during adolescence (Pattwell et al., 2012a; Hare et al., 2008; Kim

et al., 2009; Kim et al., 2011; Milad & Quirk 2012). Diminished fear extinction is thought to be a risk factor for anxiety and stress related disorders. Thus emerges the paradoxical situation in which the most common behavioral treatment approach for anxiety and stress-related disorders in adolescents is built on the same learning process that mediates these individual's vulnerability to clinical anxiety in the first place. This suggests that extinction-based therapies may be less effective for adolescents (Drysdale et al., 2013) and that alternative or optimized treatments that bypass the need for fear regulation circuitry may be more effective.

Recent studies have shown that an alternative method for attenuation of fear memories is that of memory reconsolidation (Schiller et al., 2010a; Schiller et al., 2013; Agren et al., 2012, Monfils et al., 2009). Memory reconsolidation is based on the notion that memories are dynamic rather than stable (Sara et al., 2010; Misanin et al., 1968). Every time a consolidated memory is retrieved, it returns to a fragile state and must restabilize again before becoming a stable memory (Nader et al., 2000; Dudai 2006). The temporal window of increased plasticity during which a memory undergoes reconsolidation begins approximately 10 minutes after memory retrieval and continues for at least one hour (Monfils et al., 2009), presenting an opportunity during which the memory can be updated and altered. Rodent (Nader et al., 2000; Monfils et al., 2009) and human imaging studies (Schiller et al., 2013; Agren et al., 2012) suggest that reconsolidation of fear memory is primarily mediated by the amygdala, rather than prefrontal circuitry. These findings suggest a plausible way in which adolescents may be able to overcome fear memories via interventions that alter memories within the amygdala.

In the current study we used a behavioral method employed in human adults (Schiller et al., 2010a; Agren et al., 2012; Schiller et al., 2013) and in rodents (Monfils et al., 2009; Baker et al., 2013) to test whether fear memories could be attenuated in human adolescents. We hypothesized that adolescents who received a reminder cue 10 minutes prior to extinction training would (1) show less fear recovery relative to adolescents who only received extinction training (no reminder cue); and (2) look similar to adults who received the reminder cue. This hypothesis was based on evidence of adolescents having diminished prefrontally-mediated extinction learning (Pattwell et al., 2012; McCallum et al., 2010) and of reconsolidation update altering the memory at the level of the amygdala, rather than the prefrontal cortex (Schiller et al., 2013; Agren et al., 2012; Monfils et al., 2009; Nader et al., 2000).

Methods

Participants

Seventy-four of 128 participants completed the study including 38 adolescents aged 12 to 17 (extinction condition: n=19 (11f), mean age = 14.6, sd = 1.8, mean Tanner staging = 3.98, sd = .87, range 2-5; reconsolidation condition: n=19 (9f), mean age = 14.7. sd=1.6, mean Tanner staging = 3.89, sd = .56, range = 2-5) and 36 adults aged 18 to 32 (extinction condition: n = 18 (9f), mean age = 24.7, sd = 3.6; reconsolidation condition: n = 18 (9f), mean age = 24.4, sd = 4.3.) The two adolescent experimental groups did not differ in pubertal development as indexed by Tanner pubertal staging (Fisher Exact Test, p = .09). Seven of the adolescents had a Tanner stage of 5 (Extinction group: 6; reconsolidation update group: 1). The distribution of the sexes by age group

and experimental condition were: Adult, extinction: n=18 (9f); Adult, reconsolidation: n = 18 (8f); Adolescent, extinction: n=19 (11f); Adolescent, reconsolidation: n = 19 (9f). Fifty-four (54) participants (33 adults, 21 adolescents) were excluded from analysis because they failed to show a reliable SCR and/or fear acquisition (magnitude of SCR to the CS+ was not greater than responses to the CS- during acquisition or during the first block of extinction (n = 47, 30 adults, 17 adolescents) or they failed to complete the 3-day study due to attrition (n = 7, 3 adults, 4 adolescents)). All participants were screened for exclusionary criteria, including hearing impairment, color blindness, diagnosed animal phobias and neurological and psychiatric disorders, as well as provided written consent prior to the experiment. Trait anxiety was measured by using the Spielberg State-Trait Anxiety Inventory Trait subscale (STAI-T; Spielberger, C.D., 1983) in light of evidence suggesting a negative correlation between trait anxiety and fear extinction learning (Indovina et al., 2011; Lissek et al., 2005). Trait anxiety ratings were not available for 3 adolescent participants. There was no effect of age group [F (1, 67) = .13, p =.71], experimental condition [F (1, 67) = .03, p = .86] or interaction between age group and experimental condition [F (1, 67) = 2.73, p = .10] on trait anxiety. Pubertal development for adolescent participants was measured by self-report or parental report using two standardized scales (Petersen et al., 1988; Tanner, 1962). Tanner staging correspondence ranged from 2 to 5 (mean = 3.9, sd = .72). The two adolescent experimental groups did not differ in pubertal development (Experimental group: mean = 3.98, sd = .87, range = 2 - 5; Reconsolidation update group: mean = 3.89, sd = .56, range = 2-5) All three sessions (days) of the experiment occurred within two hours of the original testing session for each participant. Participants were tested between 10 AM to 6

PM. There were no significant differences in time of day at which experiments were performed by experimental condition [F (1, 73) = 1.055, p=.426], age group [F (1, 73)= 1.456, p = .128], nor for age by experimental group [F (1, 70) = .307, p=.581].

Experimental Procedure

We utilized a differential fear-conditioning paradigm with partial reinforcement that took place over three days in two different visual contexts (Figure 1). Visual contexts consisted of two different scenes, a bedroom and a kitchen, created using 3D design software (Google Sketch-Up 2008, Mountain View, CA). Conditioned stimuli consisted of two colored windows (blue and yellow) embedded within each visual context. The unconditioned stimulus was a hybrid consisting of validated, negatively valenced animal pictures (Lang et al., 1999) and a validated aversive sound (Levita et al., 2009). The IAPS pictures used as UCSs were of threatening animals (IMG #s 1052 (fanged snake), 1120 (fanged snake), 1200 (spider), 1201 (spider on shoulder), 1205 (spider), 1300 (snarling dog), 1302 (snarling dog), 1932 (shark). Visual contexts (Context A and B), stimuli and script orders were counterbalanced across subjects. Prior to starting the experiment, participants were randomly assigned to either the reminder (reconsolidation update) or no reminder (extinction) conditions and told they should "pay close attention to everything they see and hear and understand the relationship between all of these things."

For each experimental session, stimuli were presented in a pseudo-randomized order, defined by no consecutive CS+USs during conditioning and no more than three consecutive squares of the same color in any session. Acquisition, extinction and re-extinction were each run as one continuous session, lasting 12m, 24s (conditioning,

experimental day 1) and 12m, 16s (extinction and re-extinction, experimental days 2 and 3). For the acquisition phase of the experiment, the context was presented for 3s (with a black background in the window frame where the cue will be presented), followed by stimulus presentation for 7s and presentation of the US for 1s. The timing of the trials for the extinction and re-extinction phases was identical to that of the acquisition phase with the exception of no presentation of the CS+US. The intertrial interval (ITI) for all phases was 13s, providing sufficient time for SCR to return to baseline. On experimental day 1, one colored shape (the CS+) was paired on 50% of the trials with a compound aversive stimulus (unconditioned stimulus, CS+US) within Context A. The US consisted of an aversive sound presented simultaneously with an aversive picture. Each presentation of the US consisted of the same sound and a different picture. The other colored shape (CS-) was never followed by the aversive stimulus. Partially pairing the CS+ with the tone allowed us to isolate and analyze responses to the CS+ independent of responses to the US. Participants were presented with 32 trials on Day 1 (8 CS+US, 8 CS+, 16 CS-). Twenty-four hours later, participants returned for experimental day 2. Prior to starting, participants were instructed they "may see scary pictures and/or hear annoying sounds again" and reminded to again "pay close attention to everything they see and hear." Participants in the extinction condition started with a 10-minute rest period, in front of the test computer. This was followed by a 32-trial extinction session (16 CS+, 16 CS-) in Context B. Participants who were assigned to the reconsolidation update condition received a single presentation of the conditioned stimulus (in context B), unpaired with the aversive stimulus, prior to the 10-minute rest period. These participants received one less CS+ trial during the extinction session (15 CS+, 16 CS-) in order to match the total

number of CS+ trials across experimental conditions. All participants viewed a cartoon video of Tom and Jerry (Warner Brothers) during the 10-minute break, presented on the same computer screen on which they viewed the experiment. Twenty-four hours later, participants returned for experimental day 3. Participants were instructed similarly as they were prior to experimental day 2. Participants then received a single presentation of the US, unpaired with the conditioned stimulus (reinstatement). This was followed by a 32-trial re-extinction session (16 CS+, 16 CS-). The first 2 trials were either CS+, CS- (script order 1) or CS-, CS+ (script order 2).

Physiological Measurement and Analysis

Skin conductance response (SCR) was acquired using disposable snap electrodes pre-gelled with isotonic gel, which were attached to the distal phalanx of the second and third digits of the left hand. The signal was recorded and amplified using a skin conductance recording system (MP35; Biopac) in combination with AcqKnowledge software (Biopac). E-prime software (Psychology Software Tools) was used to control the presentation of visual and auditory stimuli and send time markers to the skin conductance recording system for each context and stimulus onset/offset. The SCR was sampled at 200 Hz with a 1-HZ filter applied. SCR was analyzed manually. For each individual subject, data were smoothed. Measurable peaks were identified as the first SCR response that occurred within .5-4.5 s following stimulus onset as defined by the difference between trough and peak being equal to or greater than .02 uS (microsiemens)(Schiller et al., 2013). A zero value was added into the analysis when no peak was detected. SCR scores were square root-transformed to normalize the

distribution and were then scaled to the participant's largest response to the CS+US during acquisition to normalize responses across participants. These SCR scores were averaged for each participant for each stimulus type separately. All SCR responses reported reflect differences between responses to the CS+ and corresponding CS-.

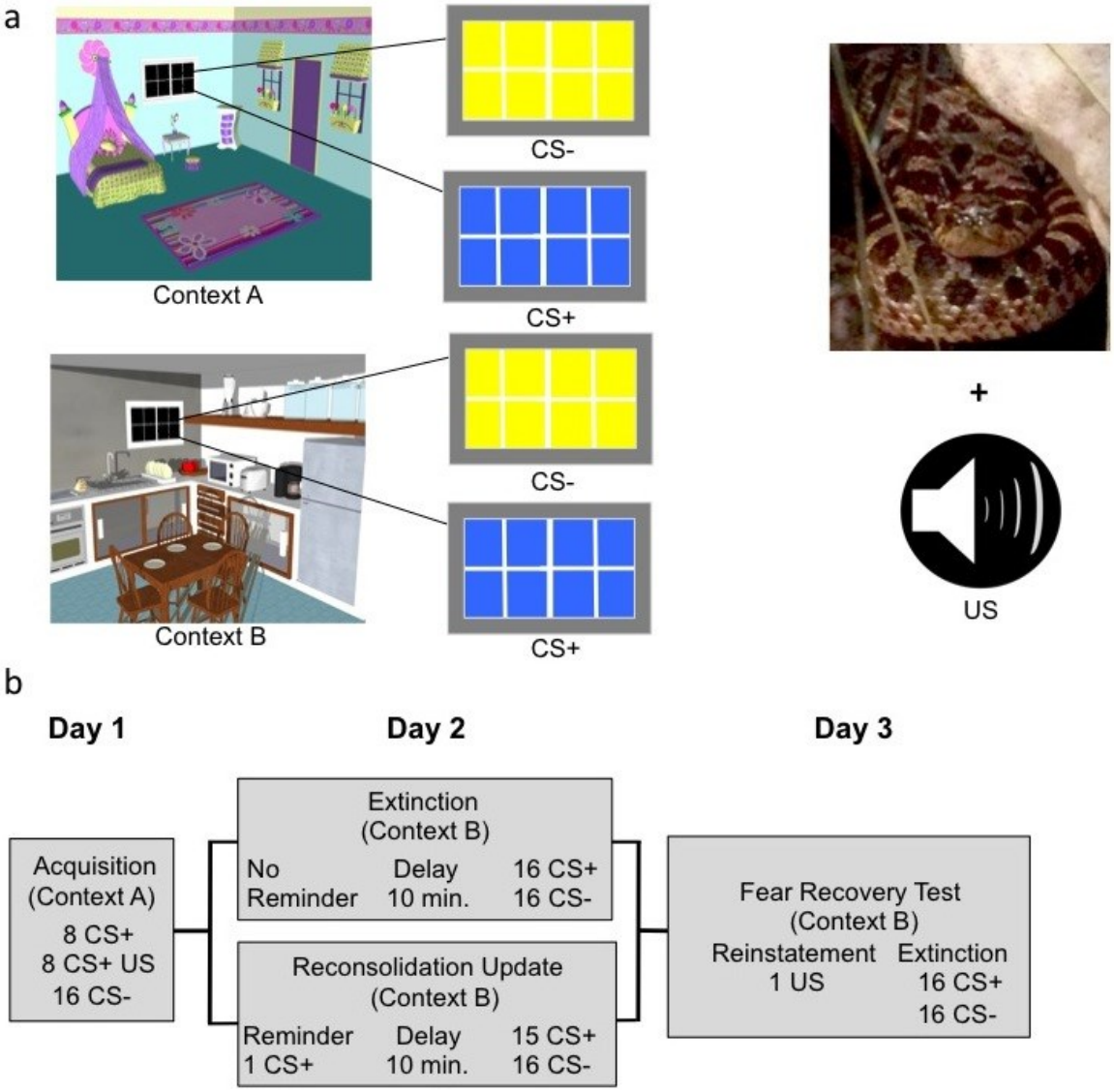

Figure 4.1. Experimental stimuli, design and timeline. (a) Contexts (A & B) were pictures of one of two rooms (kitchen, child's room) appearing on the computer screen. Conditioned stimuli (CS- & CS+) were yellow and blue windows (counterbalanced) in the rooms. The unconditioned stimulus (US) was a hybrid with visual (scary animal picture) and auditory (aversive noise) components. **(b)** Participants underwent acquisition on experimental day 1, extinction or reconsolidation update on experimental day 2 and fear recovery test (re-extinction) on experimental day 3 (All images by D.C. Johnson)

Results

The results from the acquisition phase are shown in Fig. 4.2A. Adolescents and adults showed equivalent fear acquisition across age groups [$F(1, 70) = .956$, $p = .33$] and across experimental conditions [$F(1, 70) = .23$, $p = .64$]. There was only a main effect of stimulus type [$F(1,70)=54.78$, $p < .0001$] indicating greater SCR to the CS+ than the CS- ($t(73) = 7.52$, $p < .0001$) across all participants. These results confirm that participants learned to distinguish between the CS+ (threat cue) and the CS- (safety cue). Thus subsequent group effects are unlikely due to group differences in reactivity to specific stimulus categories or strength of conditioning.

Similar to our previous findings, adolescents showed diminished extinction learning over time relative to adults as indicated by an age x time interaction [$F(1, 70) = 3.91$, $p = .05$]. Post hoc t-tests revealed a significant decrease in the mean SCR difference [(CS+) – (CS-)] score from early to late trials during extinction learning for the adults [$t(35) = 4.34$, $p < .0002$] but not for the adolescents [$t(37) = 1.78$, $p=.08$] (Fig. 4.2B). This pattern of attenuated fear extinction learning in adolescents compared to adults is in concordance with our previous findings in humans and rodents (Pattwell et al., 2012).

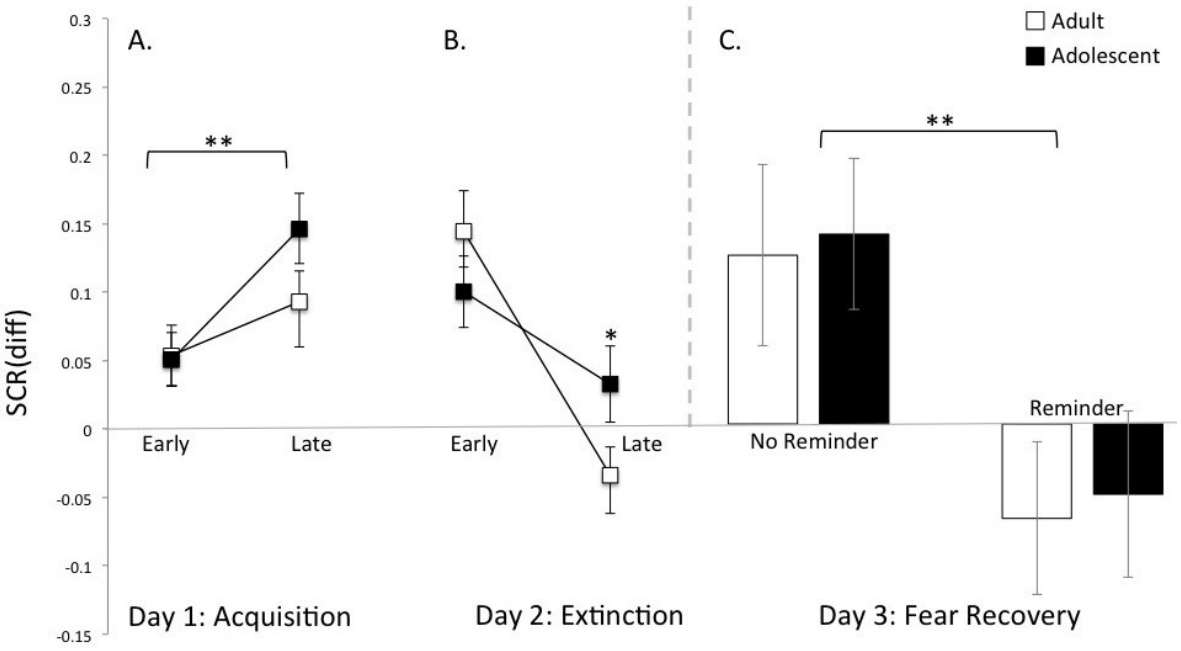

Figure 4.2. Acquisition, extinction and recovery of fear memory by age group. (A) There were no differences in differential skin conductance response (SCR) of the CS+ and CS- by age group during acquisition [$F(1, 70) = .979$, $p=.33$] and a main effect of time [$F(1, 70) = 4.564$, $p = .036$] in the mean SCR difference (CS+ - CS-) score from early to late trials. (B) There was an interaction of age group x time in extinction learning as indexed by differential SCR of the CS+ and CS- [$F(1, 70) = 3.913$, $p = .05$]. Post hoc t-tests showed significant within-session extinction learning for adults ($t = 4.34$, $p < .0002$) but not for adolescents ($t = 1.78$, $p = .08$). (C) Diminished fear memory with reconsolidation update. Participants who were reminded of the conditioned stimulus 10 minutes prior to extinction showed no recovery of fear 24 hours later, as indexed by SCR responses to the first CS+ trial of re-extinction (experimental day 3). There was a main effect of experimental condition [$F(1, 70) = 6.263$, $p = .015$] and no age group x experimental condition interaction [$F(1, 70) = .002$, $p = .966$]. All results are presented as a mean ± SEM. *$p = .05$, **$p < .05$.

Following reinstatement (isolated presentation of the US), adolescents and adults who received the reminder cue 10 minutes prior to extinction learning on the previous day, showed a diminished fear response. Conversely, adolescents and adults who did not receive a reminder cue showed robust recovery of the fear memory as indicated by the main effect of experimental condition (Figure 4.2C) [$F(1, 70) = 11.72$, $p < .001$]. These

data suggest that reinstatement of fear after extinction training can be attenuated if extinction occurs within the temporal window of reconsolidation.

Discussion

The results suggest that reconsolidation update attenuates fear recovery in adolescents similar to adult humans (Schiller et al., 2010a, Agren et al., 2012; Schiller et al., 2013; Steinfurth et al., 2014; Liu et al., 2014). While extinction learning involves the encoding of a new competing memory that leaves the original fear memory intact (Milad & Quirk 2012), the current results suggest that the safety information provided during post-retrieval extinction is integrated into the original fear memory altering its affective value even in adolescents who show diminished extinction learning.

These findings are promising in that they suggest fear extinction learning, a form of fear regulation dependent on strong functional connectivity between the vmPFC and the amygdala (Milad & Quirk 2012), is not the only means by which adolescent humans can regulate fear. Unlike other forms of memory with more diffuse neural representations (Squire & Knowlton 2000), cued fear memories are thought to be stored in the amygdala (Schafe et al., 2005). In concordance with these data, recent human fMRI studies have shown little if any involvement of the vmPFC in extinction following reconsolidation update (Schiller et al., 2013; Agren et al., 2012), suggesting that reconsolidation of fear memories occurs independent of vmPFC activation in humans. These findings could explain why reconsolidation successfully blocked fear recovery in the adolescents in the present study. While this hypothesis is consistent with the human adult imaging findings

(Schiller et al., 2013; Agren al., 2012), further studies would be required to test the neural correlates of the behavioral effects we report here for adolescents.

Contextual vs. Cued Fear

A cued fear memory can return after extinction if the conditioned stimulus is encountered in the original conditioning context or in a novel context (Bouton et al., 2004). As noted, we employed a virtual context manipulation in this study whereby acquisition occurred in context A, and extinction (with or without pre-extinction retrieval) and re-extinction occurred in context B (A-B-B) (Milad et al., 2005). This manipulation was used so measures of the CS-US association could be assessed during extinction and re-extinction, independent of the expression of contextual fear. However, all three days of this study were carried out by the same experimenter, in the same room, laboratory, building and institution. These elements constitute salient dimensions of the experimental context, which is formed by binding all of the contextual elements present during the study into an integrated and coherent representation (Maren et al., 2013). It could be argued that the reminder trial, extinction and re-extinction phases of this study occurred in a hybrid context that contained substantial elements of the acquisition context, suggesting some level of contextual ambiguity may need to be resolved in order to correctly assess the affective value of the conditioned stimulus. Evidence suggests that the process of disambiguating the affective value of discrete cues through learning and remembering contextual information is a hippocampal-dependent process (Phillips & LeDoux, 1992; Selden et al., 1991; Kim & Fanselow, 1992; Frankland et al., 1998; Blair and Fanselow, 2014). Although a full discussion of the role of the hippocampus in

updating fear memories during reconsolidation is beyond the scope of this manuscript, it is possible that the context manipulation employed in this study may have led to the recruitment of neural regions, such as the hippocampus, that are distinct from the neural correlates of reconsolidation update reported in recent human imaging studies that have maintained the same context across all experimental phases. (Schiller et al., 2013; Agren et al., 2012). Furthermore, it's possible that context played some role in mediating the effect of reconsolidation to block the recovery of fear in this study. One recent study in adolescent mice showed that attenuation of fear after reconsolidation update only occurred when a retrieval cue was presented in the acquisition context and not in a novel context (S. Pattwell, F. Lee, unpublished data, 2015), while this effect was not present for adult rodents. These findings are congruent with the results of the present study, as the retrieval cue was presented in a context that was substantially similar to the acquisition context. The aforementioned findings from Pattwell and Lee represent the possibility of a developmentally-mediated boundary condition on the reconsolidation update effect: the context in which a retrieval cue is presented could affect whether or not reconsolidation interference of cued fear memory is possible during adolescence. Future studies that employ more potent context manipulations will be needed to test whether the context in which the retrieval cue is presented can render reconsolidation update less effective, or prevent the effect from being induced at all, in adolescent humans.

Within vs Between Session Extinction

It should be noted that evidence of the persistence of reconsolidation update to attenuate fear, as indexed by the long-term fear recovery test (e.g. one year later (Schiller

et al., 2010a)) is not available for the current study. Instead, we utilized reinstatement in this study, thought to be a potent assay by which to evoke the return of conditioned fear (Schiller et al., 2010a). Conditioned fear did not return in the reconsolidation update group after reinstatement, even in our adolescents who showed diminished within-session extinction, highlighting the robustness of the effect. However, adolescents who showed diminished within-session fear extinction learning compared to adults and received no reminder cue, showed similar between-session extinction (fear recovery) following reinstatement as adults (Fig 4.2C). It is important to distinguish between within-session extinction, which refers to decreases in fear response during the extinction session, and between-session extinction, which refers to the retention of that extinction learning when presented with the same CS at a later occasion (usually 24 hours). Between-session extinction may map more directly onto clinical models of relapse after exposure therapy than within-session extinction (Milad et al., 2009). Reinstatement was utilized in this study because it is the most potent assay by which to evoke the post-extinction return of conditioned fear and therefore a strong test of the reconsolidation effect (Schiller et al., 2010a). However, it may not be the ideal assay to test for developmental or clinical differences in between-session extinction retention. Future studies that test adolescent-specific differences in between-session extinction using a milder assay such as spontaneous recovery (passage of time) rather than reinstatement (single or multiple presentation of the unconditioned stimulus prior to the fear recovery test) to evoke the return of fear might provide a more sensitive test of developmentally-mediated differences in extinction retention and would help to constrain our findings.

While exposure-based therapies are effective in the treatment of anxiety and stress disorders (McNally et al., 2005), it has been noted that as many as 40-50% of young people fail to fully benefit from such therapies (Walkup et al., 2008). The efficacy of these treatments may be impacted by age. Our data highlight how modifying the timing of therapeutic sessions based on principles of memory reconsolidation could lead to more effective attenuation of conditioned fear in adolescents (and adults). A modified version of an exposure-based CBT protocol based on memory reconsolidation might involve reminding patients of why they are there when they first arrive at the clinician's office (i.e., reminder cue), then establishing a safe and positive rapport for approximately 10 minutes (i.e., waiting for reconsolidation window) before initiating desensitization with exposure therapy. The findings may also explain why exposure-based CBT is effective for some adolescent patients and not others, and for some clinicians more than others. It's possible that positive treatment outcomes, in some cases, have been achieved through modified exposure-based CBT protocols that inadvertently capitalized on the principles of memory reconsolidation. These data provide evidenced-based support for this approach. Validating such protocols would be an important next step in establishing the utility of modified clinical therapies for adolescents based on the principles of memory reconsolidation.

Chapter 5

A personalized approach to treating fear-related disorders: Limitations, clinical implications, future studies and conclusions

This thesis has identified two factors associated with diminished fear extinction learning in humans: the developmental stage of adolescence (Chapter 2 and 4) and genetic variation in FAAH (Chapter 3). These findings suggest age and genetic background can increase any given individual's risk for developing fear-related disorders and diminish responsiveness to exposure-based cognitive behavioral therapy that build on the principles of fear extinction. The clinical relevance of these findings is that these factors (as well as additional factors such as experiential history and sex), and their various interactions, could be used as diagnostic criteria to identify patients with stress and anxiety disorder who may not respond well to extinction-based therapy (i.e. precision medicine). For these patients, alternative methods might be considered. Evidence presented in this thesis (Chapter 4) shows that a method based on the principles of memory reconsolidation enhances fear regulation. Novel or modified clinical therapies based on these findings could lead to enhanced treatment outcomes for individuals diagnosed with stress and anxiety disorder.

While this data suggests that developmental stage and genetic background can mediate fear regulatory capacity, in order for these factors to function as effective diagnostic markers, their role in mediating treatment effects of exposure-based CBT should be demonstrated. Furthermore, while converging evidence suggests that fear regulation can be enhanced by updating a fear memory during the temporal window of

reconsolidation, several studies have failed to replicate the pre-clinical findings by Schiller et al. (2010a) and others. Identifying factors that prevent or minimize reconsolidation update of fear memory will be important in order to successfully translate the pre-clinical findings into clinical application. In this chapter, I present data suggesting that age and genotypic variation mediate exposure-based CBT outcomes. I then highlight two additional factors – experiential history and sex – that may play an important role in mediating fear regulatory capacity for any given individual across development. Next I present four methodological factors – the specific category of the conditioned stimuli, online self report of anticipated fear, acquisition memory strength and age of the fear memory – that might explain some of the discrepancies seen across reconsolidation update studies and suggest potential clinical implications. Next, I present behavioral findings that suggest some non-reconsolidation based methods that go beyond standard exposure based therapies and could lead to enhanced fear regulation. I conclude by discussing future studies and the broader implications of this work.

The effects of developmental stage and heritable background on CBT treatment outcomes

While the studies presented in this manuscript suggest that developmental stage and genotypic variation play an important role in affecting fear regulatory capacity, there is also evidence that suggests these factors play a role in mediating treatment outcomes to exposure-based CBT.

Implications of developmental stage for treatment response to exposure-based therapy

Given the converging evidence of diminished extinction learning during adolescence, it may follow that exposure-based forms of therapy may be less effective during this phase of development. The notion of an adolescent-specific diminished effect of exposure therapy was recently examined in existing clinical outcome data from a randomized placebo-controlled trial of children and adolescents with anxiety (Walkup et al., 2008; Drysdale et al., 2013). Specifically this study examined the effect of CBT compared to placebo on changes in anxiety symptoms after 12 weeks of CBT or placebo as a function of age. Figure 5.1.A below shows effect sizes of CBT relative to placebo separately for children and adolescents. The effect size for adults was estimated from outcome measures (improvement in anxiety symptoms) of a comparable adult clinical trial study comparing CBT vs. placebo (Davidson et al., 2004). These comparisons reveal a non-significant trend of diminished treatment efficacy for adolescents relative to either children or adults (Figure 5.2.A). A recent meta-analysis of 16 clinical studies (Bennett et al., 2013) shows a similar, but non-significant, dip in CBT efficacy for adolescents aged 12 to 15 years compared to younger children and older adolescents (Figure 5.1.B). Collectively, these data are concordant with pre-clinical data showing diminished extinction learning in adolescent mice and humans compared to adults and pre-adolescents (Figure 5.2.A, B). Furthermore, these results provide preliminary evidence that diminished extinction learning mediated by age may be driving increased negative treatment effects of exposure-based CBT.

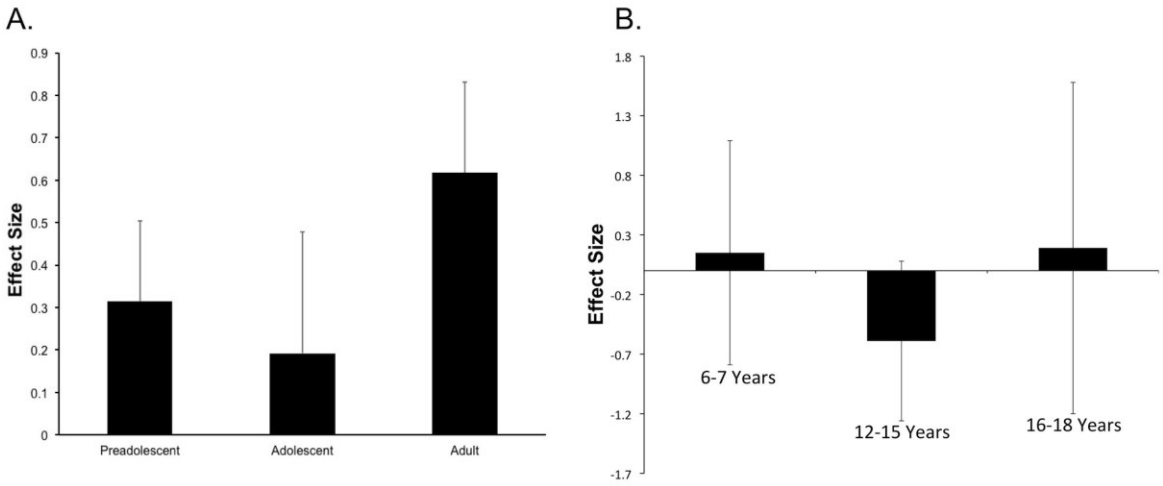

Figure 5.1. Developmental effects of CBT on anxiety symptoms.
(A) Adolescents showed a trend toward diminished treatment effect size after CBT compared to preadolescents or adults in anxiety symptoms (Drysdale et al., 2013) (B) Results from a meta-analysis (Bennett et al., 2013) showed a non-significant trend for diminished treatment effects of CBT in adolescents aged 12 to 15 years as compared to younger and older individuals. Y-axis indicates magnitude.

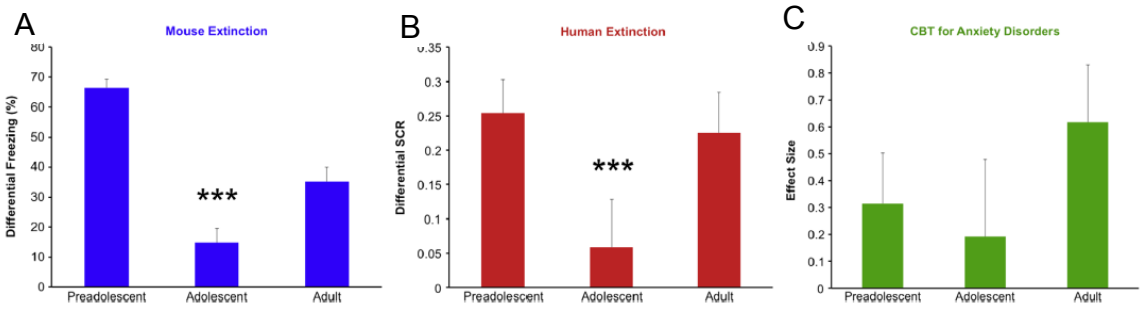

Figure 5.2. Fear extinction learning and improvement of anxiety by age.
(A) Adolescent (P29) mice show diminished extinction learning relative to adults (P70) and preadolescents (P23). (B) Human adolescents (mean age: 13.9 ± 1.47 years, n = 25) show diminished fear extinction learning as indexed by skin conductance responses (SCR) compared with adults (mean age: 22.8 ± 2.57 years, n = 28) and children (mean age: 8.8 ± 1.78 years, n . 30). (C) A similar pattern emerges when the effect size of cognitive behavioral therapy (CBT) relative to placebo is calculated separately for children (CBT: children, mean age: 9.46 ± 1.36 years, n = 90; adolescents, mean age: 14.34 ± 1.74 years, n = 49; placebo: children, mean age: 9.20 ± 1.31 years, n = 50; adolescents, mean age: 14.55 ± 1.64 years, n = 26) and adults. All results presented as mean ± SEM. ***p < .001 compared with other groups.

These studies suggest it may be important to consider the age of the individual when prescribing exposure-based treatment, but don't provide definitive support for such a claim. Across all the clinical trials reviewed, the forms of CBT used varied and often combined coping strategies and patient-focused activities with exposure. To test the premise of age-dependent effects of exposure therapy based on principles of extinction, it would be necessary to test the effects of CBT specifically focused on exposure therapy rather than on the effects of CBT that rely more on coping strategies or a combination of coping and exposure-based approaches.

Pre-clinical and clinical evidence suggests diminished fear regulatory capacity during the developmental stage of adolescence. Within the developmental and adult samples, however, there exists significant variability that can't be explained by age. In the next section, I discuss data suggesting genotypic variation as a factor that may account for some of this variability.

Implications of genetic findings for treatment response to exposure-based therapy

Pre-clinical data presented in Chapter 3 and described in Chapter 1, suggest genotypic variation in the genes FAAH and BDNF mediate fear extinction learning capacity. This data suggests that variation in these genes could be associated with treatment effects of exposure-based CBT. There are not currently any published studies we are aware of that have examined the effect of genotypic variation in FAHH on response to exposure-based CBT. Converging evidence from animal and human studies points to diminished fear extinction learning for FAAH non A-allele carriers compared to the A-allele carriers (Chapter 3, "Parallel effects of genetic variation in endocannabinoid

signaling on frontolimbic circuitry and function in adult humans"), suggesting that non A carriers would show less improvement after exposure based CBT compared to the A carriers, but this hypothesis has yet to be tested. Proof of concept for this approach has been supported, however, by a study of the effect of genotypic variation in BDNF on CBT treatment outcomes (Felmingham et al., 2013). In concordance with evidence that BDNF Met allele carriers show diminished fear extinction learning (Soliman et al 2010), this study demonstrated that exposure-based forms of CBT may be less effective for individuals with this genotype. Felmingham et al. (2013) examined the efficacy of CBT in adults with PTSD in an 8-week program of once-weekly 90-minute CBT sessions based on their genotype. Treatment response was assessed within 2 weeks of the cessation of treatment. BDNF Met allele carriers had poorer responses to exposure therapy than non-Met carriers (Figure 5.4).

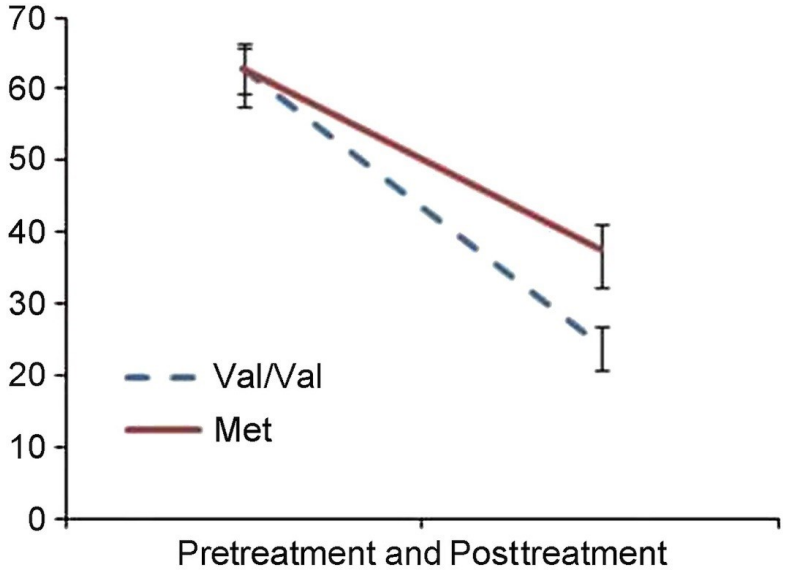

Figure 5.3. Effect of BDNF genotype on response to CBT in PTSD patients.
Met carriers showed less improvement after 8 weeks of CBT compared to non-Met carriers, as indexed by pre and post-treatment scores on the Clinician Administered PTSD Scale (from Felmingham et al., 2013).

Based on the preliminary data, it would be reasonable to hypothesize that, similar to BDNF, genotypic variation in FAAH might mediate exposure-based CBT effects in dose dependent fashion, with homozygous A-allele carriers showing more positive treatment outcomes than heterozygous A-allele carriers, and non A-allele carriers showing the least positive outcomes. There also exists the intriguing possibility of interactive and dose-dependent effects of genotypic variation in BDNF and FAHH on fear extinction learning. If the effects of variation in BDNF and FAAH on extinction learning interact in additive fashion, than the strongest extinction learning should be observed in homozygous A-allele FAAH/ non-met allele BDNF carriers, with the weakest extinction learning observed in the non-A-allele FAAH/homozygous met allele BDNF carriers. This idea is promising but has yet to be tested in a pre-clinical population or through genetic mutation studies in animals, which will be necessary before confident predictions can be made about interactive effects on exposure-based therapy outcomes. Studying the interactive effects of BDNF x FAAH on extinction learning is important as this work may one day set the stage for a diagnostic tool that predicts treatment outcomes more powerfully than either gene alone.

Additional factors that could mediate individual differences in fear regulation

Beyond genetic background and developmental history, other important individual difference factors have been associated with fear regulatory capacity and could play important roles in mediating the efficacy of exposure-based therapy: experiential history and sex.

Experiential factors impacting fear regulation

Early life stress (ELS) such as abuse or neglect is associated with a high prevalence of later psychopathology and diminished emotion regulatory capacity (Green et al., 2010; Shonkoff et al., 2009). One extreme example of early life stress is that of orphanage rearing, in which children are subjected to high stress and reduced child-parent interaction compared to their non-orphaned peers. Children reared in orphanages show higher incidence of psychopathology and emotion dysregulation compared to non-orphans (Casey et al., 2005; Tottenham et al., 2010; Malter-Cohen et al., 2013 and Gee et al., 2013). However, it has been difficult to attribute these adverse outcomes directly to orphanage rearing because pre-existing conditions (e.g., prenatal exposure to substances or genetic abnormalities) could not be ruled out.

The effect of early life stress on emotion regulation and the underlying neural substrates was recently examined in parallel studies in humans and mice (Malter-Cohen et al., 2013). Mice allow for the control of environmental and genetic backgrounds that often confound naturalistic studies in humans. Children (aged 5-11) performed an emotion regulation task as part of a functional imaging study. The task was designed to assess response latency to approach recurrent neutral cues in anticipation of a rare threat cue (fearful face). Children reared in the orphanage were slower than non orphanage-reared children to approach (detect) cues in the context of impending threat. This slower response latency was paralleled by greater fMRI BOLD amygdala activity in response to threat cues. In a parallel study, pre-adolescent mice were subjected to stress by means of limiting nesting material available to the dam, while the control group's rearing

environment was not disrupted. This manipulation led to the dam spending less time with her pups to mimic aspects of orphanage care. These mice were then tested post weaning in a task conceptually similar to the human task. In order to get the mice to approach potential threat, one of their favorite cocktails of sweetened condensed milk was used. The mice were trained to obtain the milk from a nozzle over several days in their home cage (i.e., approach a cue). On the last day the context was changed to a well-lit, odor-barren novel cage, an environment of potential threat for rodents. Thus like the human, the mouse had to approach a cue (nozzle) in the face of potential threat (brightly lit, novel environment). Early-life stress altered fear regulation in the postweaned mice, as measured by longer approach latencies to the nozzle in the novel cage. This behavioral pattern was mirrored by enhanced C-Fos activity in the amygdala to threat cues in pre-adolescent mice, paralleling the human findings.

How persistent are these changes? Given that mice age more quickly than humans, the effects of early life stress in the mice were tracked into adulthood (i.e., postnatal day 70). Atypical behavior and brain activity persisted long after the stressor was removed and even with development of prefrontal regulatory regions. These results are consistent with other animal studies that have shown chronic stress exposure mediates long-term amygdala reactivity and anxiety-like behavior in adulthood (Vyas et al., 2002).

In an independent study of emotion regulation in children adopted from orphanages abroad, Gee and colleagues (2013) showed that children reared in orphanages have altered frontolimbic circuitry relative to nonadopted children. While this study did not directly test whether early life stress mediated changes in fear extinction learning per

se, it demonstrated heightened amygdala-driven reactivity to repeated presentations of empty threat. This pattern coupled with immaturity of prefrontal control regions and inputs may diminish emotion regulation and set the stage for long lasting emotion dysregulation.

Together the developmental findings from rodent and human studies highlight how early life stress can lead to emotional dysregulation and altered connectivity of frontolimbic circuitry that may increase the risk for psychopathology. Previous animal work examining the effects of early life stress (e.g. maternal deprivation) on long-term outcomes have shown altered social and fear behaviors and fronto-amygdala circuitry (Hofer, 1996; Romeo et al., 2003; Callaghan and Richardson, 2011). These findings are similar to these reports and have been extended by Gee and colleagues, (2013) who show a shift in the typical development of fronto-amygdala connectivity that is associated with anxiety-like behavior in orphanage-reared children. These findings suggest the importance of early interventions or rescue from early life stress to prevent atypical wiring of frontoamgydala circuitry that can lead to patterns of pathological fear responding. Evidence in support of early intervention is provided by findings that children adopted within 12 to 24 months of age from the orphanage environment appear more resilient than those adopted later (Tottenham et al., 2010; Rutter and O'Connor, 2004; Rutter et al., 2010; Gunnar et al., 2000, 2007; Nelson et al., 2007). The clinical implications of these findings are that ELS could persistently diminish treatment response to exposure-based CBT all the way into adulthood. This suggests alternative methods of fear regulation characterized by diminished reliance upon prefrontal mechanisms (reconsolidation update) or enhanced frontoamygdala-mediated regulation of fear via

hippocampal contributions (as depicted in Figure 5.4) might be optimal for this population.

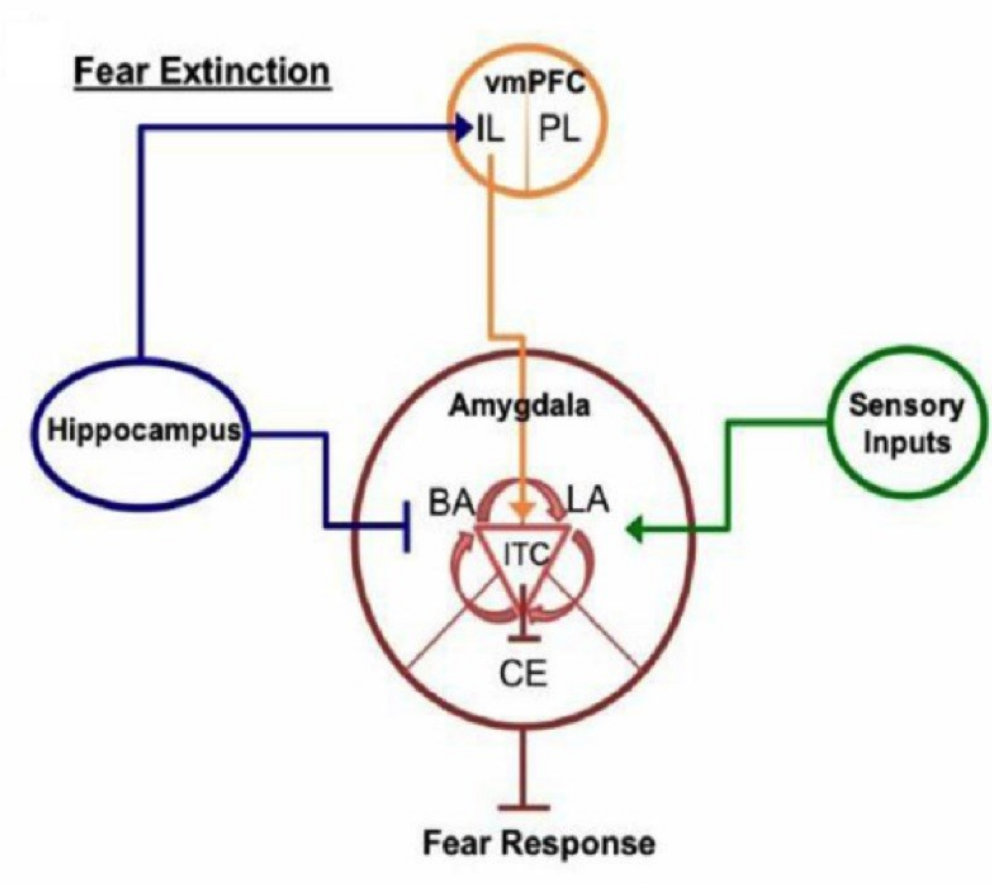

Figure 5.4. Role of hippocampus in neural circuitry underlying fear extinction learning. During extinction of conditioned fear, hippocampal projections lead to excitation of IL (vmPFC in humans) and inhibition of BA neurons. Excitatory projections from IL innervate inhibitory ITC cells in the amygdala. Convergence of ITC, BA and LA inputs during extinction blocks output of CE neurons, blocking downstream CE output and blunting fear expression. (BA, basal amygdala; LA, lateral amygdala; ITC, intercalated cells; CE, central nucleus of amygdala; IL, infralimbic cortex; PL, prelimbic cortex). (Figure from Pattwell et al., 2013)

The impact of sex differences on fear extinction learning

Studies have shown that females are at significantly higher risk for developing anxiety disorders than males (Kessler et al., 2005), and have demonstrated sex differences in neural regions associated with fear learning processes such as the amygdala, hippocampus and medial prefrontal regions (Goldstein 2001). However, these data are not congruent with preclinical studies showing no effects of sex on extinction learning (Baran et al., 2009; Baran et al., 2010). These discordant findings have been at least partially explained by studies showing that sex-based differences in fear extinction are mediated by the phases of the estrous cycle in female rats and the menstrual cycle in women (Zeidan et al., 2011; Milad et al., 2009; Milad et al., 2010). That is, sex differences in these studies are not revealed in naturally cycling females, in either the rodents or the humans; they are only revealed when cycle phase is taken into consideration. In both rodents and humans, females with low estradiol showed diminished extinction retention compared to females with high endogenous estradiol and males (Milad et al., 2009; Milad et al., 2010). This suggests that sex hormones may influence fear regulatory capacity in women. The rat estrous cycle consists of five phases: the estrus, metestrus, diestrus, proestrus and estrus phases. Naturally cycling rodents demonstrated the most robust extinction recall when they underwent extinction during the proestrous cycle (Milad et al., 2009), during which levels of endogenous estrogen and progesterone are highest. This finding suggests that these sex hormones may be facilitating enhanced extinction learning in the female rodents (Milad et al., 2009). Similarly in humans, it has been shown that women with high estrogen exhibit significantly less post-extinction fear recovery compared to women with low estrogen.

The effect of facilitated extinction recall in women with high estrogen levels has been linked to increased vmPFC, hippocampal and amygdala activation during extinction recall, providing support for the notion that estrogen may be mediating extinction learning, potentially through enhanced consolidation (Zeidan et al., 2011).

These findings carry some important clinical implications, particularly with regards to potential sex differences in response to exposure-based therapy. For one, exposure-based CBT might be differentially effective for any given woman in a time-sensitive fashion, with treatment outcomes dependent on the specific phase of the menstrual cycle during which treatment occurs (both estrogen and progesterone levels are at their lowest levels during the early follic phase of the cycle, suggesting this may be a suboptimal time for exposure-based therapy). Furthermore, endogenous estradiol levels can decrease with the use of oral and intrauterine contraceptives, as well as during postpartum periods and menopause, during which times women show heightened risk for developing stress and anxiety disorders (Altshuler et al., 1998; Schnatz et al., 2010; Schmidt & Rubinow, 2009; Harsh et al., 2009). In summary, this data suggests that timing exposure-based therapy to the menstrual cycle might be necessary to optimize treatment outcomes for women, whereas alternative therapeutic approaches might be considered when timing changes aren't possible.

Reconsolidation update of fear memory: limitations and promises

The factors described above – genotypic variation, developmental stage, experiential history and sex – may explain some of the variability observed in response to exposure-based treatments for individuals with anxiety and stress disorders (Walkup et

al., 2008), and could help clinicians identify individuals who might not respond well to exposure-based therapies. Preclinical data suggests that an approach based on the principles of memory reconsolidation (Chapter 4, "Extinction during memory reconsolidation blocks recovery of fear in adolescents") offers a promising alternative for altering or regulating fear that bypasses prefrontal regulation and could set the stage for new treatments for such individuals. In this next section, I discuss some divergent results across fear memory reconsolidation studies in humans and touch on potential limitations and challenges in translating the pre-clinical findings into new therapeutic approaches. This is followed by a discussion of alternatives to reconsolidation or exposure-based methods of fear regulation.

Methodological factors that could mediate or block the reconsolidation update effect and clinical implications

While several studies have demonstrated that extinction during reconsolidation attenuates conditioned fear in humans (Schiller et al., 2010a; Schiller et al., 2013; Agren et al., 2012; Oyarzun et al., 2012; Steinfurth et al., 2014; Liu et al., 2014), some studies have failed to replicate these results (Soeter & Kindt 2011; Kindt & Soeter 2013; Golkar et al., 2012). Methodological differences across these studies are numerous and likely play a role in these differing results. Evidence suggests that the following factors could play important roles in rendering the behavioral manipulation less effective or preventing reconsolidation from being induced at all: (1) the use of fear-relevant compared to fear-irrelevant pictures as conditioned stimuli, (2) online self report of anticipatory fear, (3) acquisition strength, and (4) the age of the conditioned fear memory. As these factors

represent potential boundary conditions on the reconsolidation update effect, it is important to consider how they might impact efforts to target reconsolidation of traumatic memory as a potential treatment for psychopathology.

Fear-relevant vs fear-irrelevant conditioned stimuli

In three of the non-replications of fear memory reconsolidation update (Soeter & Kindt 2011; Kindt & Soeter 2013; Golkar et al., 2012), fear-relevant pictures were used as conditioned stimuli instead of neutral stimuli, such as geometric shapes (Schiller et al., 2010a; Schiller et al., 2013; Johnson & Casey, 2015b). The classification of a given stimuli as "fear-relevant" is based on an evolutionary perspective that suggests our fears of certain objects, such as snakes, spiders, or angry human faces, are innate because they represented threat to the survival of our ancestors. By this account, responses to fear relevant stimuli may be qualitatively distinct from responses evoked by fear irrelevant stimuli, such as guns, motorcycles and automobiles (or neutral stimuli such as geometric shapes). Fear relevant stimuli may generate enhanced conditioning compared to fear irrelevant stimuli (Ohman & Mineka 2001), and are particularly resistant to extinction compared to neutral stimuli (Mineka & Ohman 2002), such as the colored squares used in Schiller et al. (2010a) and Johnson & Casey (2015b). This is an important methodological distinction and suggests the important clinical consideration that phobias based on innately feared objects might not be amenable to treatment via a reconsolidation update-based clinical approach. Shiban et al. (2015) tested reconsolidation-augmented exposure therapy on a clinical sample of spider phobics and showed no effect of reactivation of fear memory prior to exposure therapy. As suggested, one possible

explanation for these negative results is that phobias with fear-relevant stimuli at their core are not entirely built on associative learning processes and may be based on representations qualitatively distinct from the type of associative fear memories encoded in Pavlovian conditioning studies using neutral images as conditioned stimuli.

Online self-report of anticipatory fear

Kindt & Soeter (2013) and Soeter & Kindt (2011) failed to replicate the finding that the return of fear was prevented when extinction occurred during reconsolidation. In these studies, participants made online ratings of distress and US expectancy, respectively. The continuous evaluation of the experimental contingencies forced the participants to direct attention towards, and may have strengthened their conscious knowledge of, the CS-US relationship. This behavior may have altered the neural substrates mediating the learning (Funayama et al., 2001; Coppens et al., 2009) by recruiting higher cortical brain regions involved in declarative knowledge (Weike et al., 2007) and driving amygdala-based fear responses through top-down mechanisms even if the original association had been disrupted at the level of the amygdala during reconsolidation. In a clinical context, standard exposure-based CBT protocols often dictate that patients self-report their fear levels during the exposure session (S. Bennett, personal communication, November 15, 2014). While this may be viewed as an important means by which to gauge the within-session effectiveness of the therapy, its possible that this process could change the nature of the fear memory, shifting the neural substrate from a primarily subcortical to a more cortical representation and making the memory less amenable to attenuation via reconsolidation update.

Acquisition memory strength

There are wide discrepancies across studies in reinforcement rates used during fear acquisition. Soeter and Kindt (2011), and Kindt and Soeter (2013) used 100% and 75% reinforcement on CS+ trials during acquisition (and demonstrated no effect of reconsolidation update), while studies by Schiller et al., (2010a), Oyarzun et al., (2012) and Johnson & Casey (2015b) employed 37.5%, 37.5% and 50%, respectively. It has been suggested that the lack of disruption of reconsolidation in Soeter and Kindt (2011) and Kindt and Soeter (2013) might be attributed to stronger conditioning creating a more potent fear memory that proved resistant to reconsolidation update (Oyarzun et al., 2012; Golkar et al., 2012). However, recent findings by Agren et al. (2012) have shown persistent attenuation of fear memory utilizing a reactivation – extinction procedure with 100% reinforcement during acquisition, suggesting reconsolidation update can still occur with continuous reinforcement.

Some evidence does suggest memory strength may be an important factor in whether or not reconsolidation can be induced. Wang et al., (2009) manipulated fear acquisition strength by varying the number of reinforcement trials, with mice in the strong learning condition receiving 10 tone-shock trials and those in the weak learning condition receiving just 1 trial. While mice in the weak training group showed no return of fear after post-reactivation administration of anisomycin, mice in the strong training group showed significantly more freezing at the end of the extinction session and strong return of fear after reconsolidation interference. Wang et al. (2009) suggests that increasing reinforcement trials during acquisition down regulates mechanisms in the

lateral basal amygdala upon which reconsolidation is dependent and prevents pharmacological disruption of a fear memory during reconsolidation from occurring. In clinical terms, this represents a significant issue as it would suggest the possibility that strong trauma memories, such as those that often set the stage for stress and anxiety disorders such as PTSD, may be resistant to undergoing reconsolidation, which would potentially negate reconsolidation as a therapeutic target.

One challenge in interpreting the results of human fear learning studies in the context of real-life trauma is that, due to ethical concerns, the intensity of the US is relatively mild and "annoying but not painful" (Johnson and Casey, 2015b), which minimizes its aversive nature. Most human studies are required to push the aversiveness of the US up to this ethical ceiling in order to get sufficient fear acquisition at all, let alone different levels of acquisition strength. As such, it's difficult to know whether the strength of fear acquisition typically seen in human studies constitutes "weak" or "strong" learning relative to the rodent studies. Breaching this ethical ceiling is neither a realistic nor desirable option. Therefore, we are largely dependent, for the time being, upon rodent studies to explore questions regarding the effects of acquisition memory strength on reconsolidation processes. This highlights an important feature of a translational (animal to human) approach to understanding fear learning processes. Due to the strong evolutionary and cross-species conservation of fear learning processes and associated neural substrates, questions that are not conducive to exploration in the human species can often be usefully probed in the rodent.

Age of the conditioned fear memory

The age of a fear memory may also be an important factor in mediating reconsolidation processes. While Wang et al. (2009) showed a return of fear after reconsolidation interference in the "strong training" rodents, this condition was transient. When a strong memory was reactivated just 7 days after training, post-reactivation infusion of anisomycin did not disrupt the fear memory, whereas the same procedure conducted 30 or 60 days after training led to attenuation of the fear memory. This would suggest that a strong fear memory might be resistant to reconsolidation update during an early transient window after which the fear association is transformed and reconsolidation interference becomes possible. Steinfurth et al (2014) have demonstrated in adult humans that, similar to young memories (one day old), older fear memories (seven days old) can be altered through extinction training during memory reconsolidation. While this finding is consistent with rodent studies that have shown older memories could be altered during reconsolidation using a pharmacological approach (Nader et al., 2000; Debiec et al., 2002), it is not congruent with a behavioral study in mice that showed return of fear after extinction during reconsolidation of a 7-day old memory (Clem & Huganir, 2010). One possible explanation for these divergent results is cross-species differences in life expectancy, as 7 days in mice is roughly equivalent to 70 days in humans (Quinn 2005). Future studies that test whether longer-term fear memories, similar to acute fear memories, are amenable to reconsolidation update in humans will shed light on this issue.

This preclinical research highlights many potential factors that could prevent reconsolidation interference from occurring, suggesting potential challenges for both future basic research and development and implementation of therapeutic applications based on the principles of fear memory reconsolidation. Certain types of fear memories, such as those involving the kinds of fear-relevant stimuli that often characterize certain phobias, may be resistant to reconsolidation update. Self-report of fear during exposure therapy might change the neural representation of a fear memory, preventing it from being altered during reconsolidation. Finally, in a clinical setting, the issues of strength and age of a fear or trauma memory are important because patients presenting with anxiety and stress disorders have often experienced extremely aversive past events, one or multiple times, that may have occurred anywhere from days to months to years in the past. Relatively few studies have tested the impact of these factors on reconsolidation update in humans. Future work that tests the temporal boundaries of reconsolidation, as well as whether memory strength presents a boundary condition on reconsolidation update, will be useful in determining when and under what conditions a reconsolidation update-augmented therapeutic approach may be appropriate. Despite these challenges, reconsolidation augmented approaches to treating fear-related disorder holds significant promise and efforts are underway to test reconsolidation-augmented clinical protocols (Shiban et al., 2015). Although outside the realm of fear learning, Xue et al (2012) has provided an instructive example of research based on the principles of memory reconsolidation that begins to bridge the gap between the lab and the clinic. In a series of rodent and human studies, they utilized a memory reactivation – extinction procedure to decrease drug effects and drug seeking in a rat model of relapse, and reduce drug

cravings for in-patient heroin addicts. Multiple clinical trials testing reconsolidation-augmented therapies are underway, including studies in which reconsolidation has been targeted to reduce fear of flying for patients with flying phobias and treat anxiety disorders, as well as to test novel interventions for cocaine and nicotine dependence (https://clinicaltrials.gov/ct2/results?term=reconsolidation&Search=Search. Accessed January 12, 2015).

Other promising non-pharmacological approaches to augmenting extinction

Cognitive forms of fear regulation

While the efficacy of exposure-based CBT depends on the ability of a given individual to acquire and retrieve extinction memories (Berry et al., 2009), cognitive strategies can also be employed to regulate conditioned fear responding. Standard CBT protocols generally include a cognitive component in which patients are directed to strategically reframe anxiety-provoking situations in order to reduce the negative emotional responses that these situations elicit (Beck & Emery 1985). One form of cognitive regulation is emotional reappraisal, in which an automatic emotional response to an emotional event is controlled through conscious transformation of its meaning (Gross 2001). Human fMRI studies have shown that successful reappraisal of negatively valenced stimuli is dependent upon recruitment of prefrontal and cingulate regions associated with cognitive control (Ochsner et al., 2004). However, the prefrontal cortex is still maturing into early adulthood, suggesting that children and adolescents might show diminished reappraisal capacity compared to adults. Recent studies have presented some evidence to this effect, with reappraisal success positively correlating with age across

adolescence (Silvers et al., 2012; McCrae et al., 2012). Although only a small number of studies have examined the effects of developmental and individual differences on reappraisal of threat, future pre-clinical research that tests the effect of age, genes, sex and experiential history on different forms of reappraisal across childhood and adolescence will help shed light on which reappraisal strategies might be most effective at any given individual.

Attentional Control

Another important cognitive factor mediating fear regulation is attentional control. The ability to increase attention to threat stimuli is an adaptive function that facilitates the detection of danger (LeDoux 2000). However, devoting an inappropriate amount of attentional resources to non-significant or low-level threats can be maladaptive. This notion is supported by research providing strong evidence for a positive correlation between threat related attentional bias and anxiety (Bar-Haim et al., 2007; Mathews and MacLeod, 1985; Monk et al., 2008). Emerging data suggests that reductions in attentional threat bias can be achieved through attentional bias modification therapy (Hakamata et al., 2010; Bar-Haim, 2010; MacLeod and Mathews, 2012), which involves teaching individuals to shift attention away from threat-related stimuli through repetitive, computer-based training (MacLeod and Mathews, 2012). While attentional bias modification therapy has been shown to lead to decreases in threat bias as well as diminished anxiety symptoms (Hakamata et al., 2010), treatment outcomes across studies are inconsistent (Mogoase et al., 2014), suggesting further research will be

necessary in order to optimize these techniques and determine when, and for whom, attention-based therapy will be most beneficial.

Safety Signal Learning

Emerging evidence suggests that adaptive regulation of fear is not only dependent on successful extinction of conditioned fear, but also upon safety signal learning (i.e. conditioned inhibition); that is, the ability to utilize the presence of a safety cue to transfer inhibition to the aversive cue (Rescorla 1969; Jovanovich et al., 2012). In the lab, safety signal learning (i.e. conditioned inhibition) is typically assessed by training participants to pair one cue with an aversive event and another cue with the absence of the aversive event. These two cues are subsequently paired to test if participants utilize the presence of the safety cue to inhibit fear responses to the fear cue. Research exploring safety learning processes have been gaining interest, as studies have shown evidence of diminished safety learning ability in individuals with PTSD compared to control subjects (Jovanovich et al., 2009, 2010a, 2010b, 2012).

Data suggests distinct underlying neural mechanisms for safety signal learning compared to fear extinction learning. As described in detail in Chapter 1, the neural mechanisms of cued fear extinction learning consist of projections from the IL prefrontal cortex (mouse) / subgenual (human) vmPFC to the amygdala, with contextual fear regulation mediated via projections from the hippocampus to the amygdala and mPFC. While the circuitry associated with safety signal learning has been less well characterized, and much of the research record shows contrasting results, collective

evidence suggests important roles for the medial prefrontal cortex, hippocampus, striatum and insula.

While some studies have shown lesions or pharmacological inhibition of the vmPFC in rodents had no effect on the safety signal, suggesting safety signal learning may occur independent of vmPFC activation (Gewirtz et al., 1997; Christianson et al., 2008), multiple studies have shown increased activity in the vmPFC in response to cues that signal positive compared to negative outcomes (Delgado et al., 2006; Kalisch et al., 2006; Milad et al., 2007; Mobbs et al., 2010; Phelps et al., 2004; Quirk et al., 2006; Schiller et al., 2008). Furthermore, Likhtik et al. (2014) have shown increased synchrony between mPFC and the basal lateral amygdala (BLA) for both safe and aversive cues in the theta frequency (4-12 Hz) in animals who successfully discriminated between these cues (as indexed by freezing) compared to those who showed fear generalization. Thus, collective evidence suggests a strong role for the prefrontal cortex in safety signal learning.

Early theorists proposed that the hippocampus is essential for safety signal learning (i.e. behavioral inhibition; Douglas et al., 1967; Kimble et al., 1969), but subsequent research has provided mixed results. Chan et al. (2001) showed that the hippocampus was involved in retention and retrieval of safety signal but not its acquisition, while Kazama et al. (2010) conducted a hippocampal lesion study in adult non-human primates, showing that the hippocampus is not necessary for either safety signal learning or the blunted fear expression observed during the safety transfer test. It has also been suggested that safety signals may themselves constitute a form of reward.

Evidence indicates that responses in the striatum, a neural region associated with encoding reward value, were increased with safety conditioning and decreased with fear conditioning (Rogan et al., 2005). Further support for this notion is provided by a rodent study showing a large proportion of neurons in the basal lateral amygdala that respond to a CS- also are active in response to a CS predictive of reward, suggestive that safety and reward learning rely on overlapping cell populations in the amygdala (Sangha et al., 2013). Another structure that may play a central role in safety signaling is the posterior insular cortex (IC), which acts as a way station for sensory input, with direct projections to the amygdala (Nieuwenhuys, 2012). Evidence from lesion studies showed that permanently or temporarily inactivating the IC (Christianson et al., 2008, 2011) eliminated the stress-buffering effects of the safety signal. This is in concordance with data showing functional and structural abnormalities in IC in anxiety and PTSD (Paulus and Stein, 2006; Hughes and Shin, 2011).

While the neural mechanism(s) underlying safety learning are not well understood, the data outlines a neural circuit both partially overlapping yet qualitatively distinct from that associated with extinction learning, and highlights its importance as a unique and important diagnostic and therapeutic target. Among the diagnostic possibilities include the possibility that diminished or abolished safety signal learning could represent a risk factor for the development of fear-related disorders, such as PTSD (pre-trauma), or if assessed in the aftermath of trauma, could be predictive of who might develop pathological conditions (Javancovic et al., 2012).

A comprehensive discussion of alternative behavioral methods that could lead to enhanced fear regulation is beyond the scope of this manuscript. However, it should be noted that, similar to exposure-based therapies, clinical treatments based on emotional reappraisal, attentional control or safety learning approaches may be more effective for some individuals than others. It is important that future research identifies factors that mediate any given individual's capacity to successfully employ such strategies to regulate fear. Identifying these factors may suggest a personalized combination of therapies customized to the individual to maximize positive treatment outcomes.

Future Studies

This manuscript has presented pre-clinical research identifying factors associated with developmental and individual difference in the capacity to regulate fear, and also described a method based on the principles of memory reconsolidation that led to enhanced fear regulation. Clinical relevance has been a guiding principle of this work, and, as such, it is hoped that these studies represent a foundation upon which new or augmented clinical approaches to treating anxiety and stress disorders might be built. Below, I describe three current projects, designed to bridge important translational gaps and build on the work presented in Chapters 2 through 4.

Direct assessment of the relationship between fear extinction learning and CBT treatment outcomes in a clinical anxiety population

Chapter 2 ("Altered fear learning across development in humans") described that diminished extinction learning has been observed in both adolescent humans and mice

while evidence presented earlier in this chapter showed a trend for diminished treatment effects of CBT during this developmental stage (Figure 5.1). While this these data are suggestive of a relationship between extinction learning capacity and CBT treatment outcomes (Figure 5.2), direct within-subject evidence would provide crucial support for this link. In collaboration with a pediatric anxiety clinic, we are currently testing children and adolescent anxiety patients in a study that will assess the patients' fear regulatory capacity utilizing a Pavlovian fear conditioning and extinction paradigm in a controlled, experimental setting. This will be followed by 8 weeks of CBT treatment for these same patients, allowing us to directly compare the patients' extinction learning in the experimental setting with their treatment outcomes. We hypothesize that the data will show a positive correlation between extinction learning and treatment outcomes from CBT. This would provide strong evidence for diminished fear extinction learning as a mechanism underlying the diminished responses to exposure-based therapy previously observed during adolescence and would further highlight the need to develop alternatives to exposure-based therapies for this developmental group.

Probing the effect of genotypic variation in FAAH (and interactions with BDNF) on CBT treatment outcomes

Chapter 3 ("Identical genetic variation in mouse and human FAAH enhances extinction learning and frontoamygdala connectivity") presented evidence that genotypic variation in FAAH can mediate fear extinction learning. This suggests that FAAH might impact treatment outcomes from CBT, similar to that shown for BNDF by Felmingham et al. (2013). Plans are currently underway to test the prediction of improved CBT outcomes

for A-allele carriers compared to non A-allele carriers, potentially setting the stage for genotypic variation in FAAH to be used as a biomarker for response to exposure-based CBT. Furthermore, genotypic variability in FAAH and BDNF may interact to mediate fear extinction learning, leading to enhanced fear regulation when both favorable variants are present compared to either one alone. If future pre-clinical data supports this hypothesis, this would suggest that the BDNF x FAHH interaction might act as a potent biomarker for treatment outcomes from CBT.

Developing and testing a reconsolidation-update augmented clinical therapy protocol for treatment of clinical patients with PTSD

Chapter 4 ("Extinction during memory reconsolidation blocks recovery of fear in adolescents") presented data suggesting an alternative approach to fear regulation is possible based on the principles of memory reconsolidation, largely bypassing prefrontal regulation of fear, making it ideal for special populations who show diminished capacity for prefrontally-mediated fear regulation. This project plans to implement and conduct a preliminary investigation of a reconsolidation update-enhanced exposure therapy for the treatment of PTSD in military personnel. The study would attempt to fill a translational gap between preclinical findings and clinical practice by first testing the effectiveness of reconsolidation update in PTSD subjects in a controlled, experimental study, and, second, by establishing and testing a clinical protocol of reconsolidation update-enhanced exposure therapy with the goal of reducing PTSD symptoms and setting the stage for a large scale clinical trial. This study will also include age and genetic factors to allow for the testing of the effectiveness of reconsolidation update-enhanced exposure therapy on

the patient populations that would potentially benefit the most from this treatment regimen.

Conclusions

The preliminary data presented here suggest diagnostic criteria by which to identify individuals with fear-related disorders who may not respond well to exposure-based therapy by age (adolescence) and genotype (BDNF and possibly FAAH), as well as describing two additional factors that could play an important role in treatment outcomes, sex and experiential history. These findings identify factors that could serve as predictive markers of response to clinical treatment. These markers could potentially be used to help guide clinicians in identifying patients for whom more efficacious treatment approaches (such as reconsolidation-augmented exposure therapy) might be necessary and may contribute to efforts to build a personalized approach to treating psychiatric disorders built on the core premise that individual differences should play a major role in tailoring the therapeutic approach (Figure 5.5).

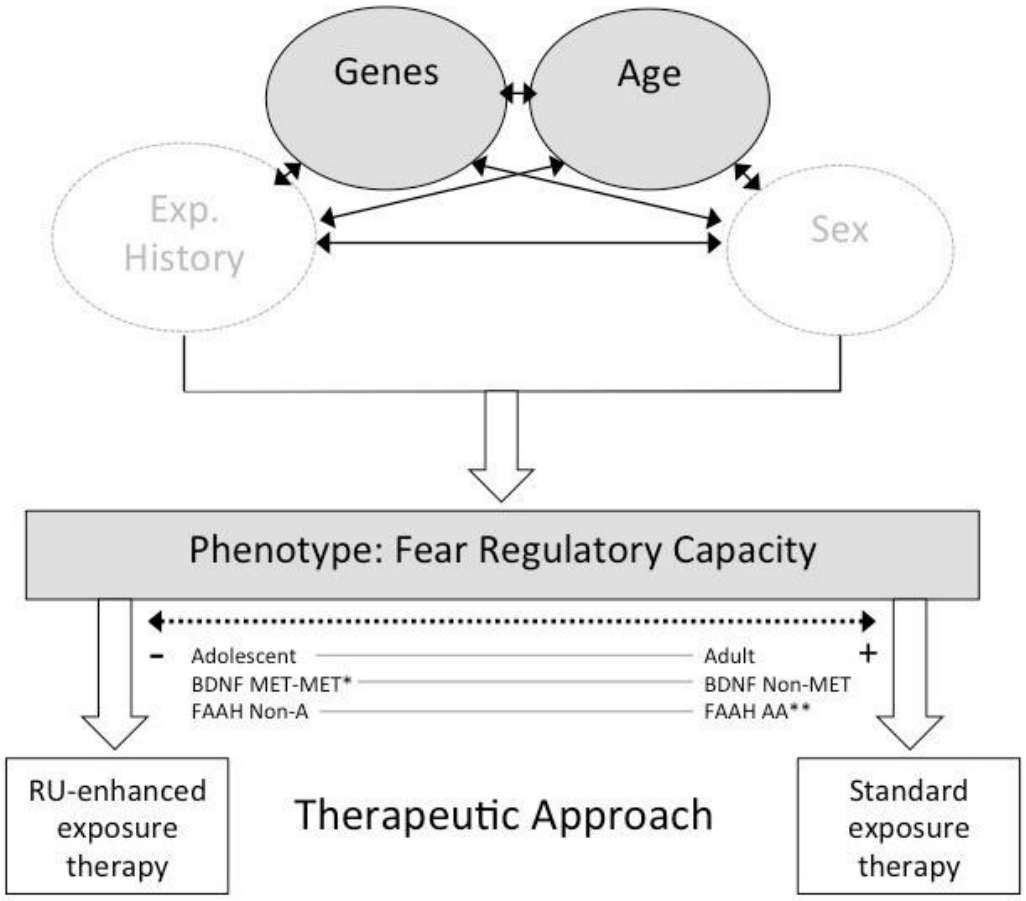

Figure 5.5. An individually tailored approach to treating anxiety and stress disorders. The top level of this schematic depicts factors that mediate fear regulatory capacity: genetic background, developmental stage, experiential history and sex. N-dimensional interactions between these factors will mediate the specific point at which any given individual falls along the phenotypic continuum. Assessing these factors may allow a mental health professional to predict whether a patient may or may not respond well to exposure-based therapy. This would allow for a personalized therapeutic approach targeted to the individual.
*Loss-of-function mutation** Gain-of-function mutation

While the pre-clinical findings presented here are promising, the perhaps even tougher road is still ahead, which is to translate these findings into efficacious clinical therapies. Translating experimental research into effective clinical treatment has historically been a slow process, as is exemplified by the case of cognitive behavioral

therapy. An empirically validated and undeniably successful behavioral treatment for fear-related disorders, exposure-based CBT is based on the general principles of classical conditioning and associative learning, originally discovered in the early 20th century by Watson and Rayner (1920), Pavlov (1927) and others. But these ideas had little impact on clinical practice during their own time, as psychiatrists were influenced almost exclusively by Freudian psychoanalysis. It wasn't until the early 1960s that therapies built on principles of associative learning emerged and overcame Freudian techniques, through the systematic application of basic learning principles discovered by Wolpe (1958) and the research and advocacy of Eysenck (1952, 1960), among others. In the decades since, clinical therapies have been continually refined by new pre-clinical findings provided during the cognitive revolution of the 1970s and 80s (Beck, 1985) and the rise of affective neuroscience research, and technologies such as fMRI, during the 1990s and 2000s (Phelps et al., 2004; Milad and Quirk, 2012). The fear domain has provided a potent example of the power of translational research, as basic animal and human research programs and findings from the fields of genetics, molecular biology, pharmacology, psychiatry and clinical psychology have mutually contributed to exponentially and rapidly increasing our understanding of fear-related behaviors and disorders, across molecular, genetic, circuit and behavioral levels.

One of the primary modern challenges to the goal of improving treatment of psychiatric disorders is derived from the high variability observed across individuals in response to treatment. A homogenous approach to treating pathological conditions such as anxiety and stress disorder is bound to be suboptimal for many patients. As the

application of learning theory once set the stage for a new and more effective therapeutic approach to treating pathological fear, delineating the individual and developmental differences that impact fear regulation may do the same, leading to identification of patients who may not respond well to standard clinical protocols and who may require a personalized treatment approach (i.e. precision medicine). This approach has been promoted by President Obama, who recently announced (and requested significant financial support for) the Precision Medicine Initiative, a government sponsored enterprise that proposes to "pioneer a new model of patient-powered research that promises to accelerate biomedical discoveries and provide clinicians with new tools, knowledge, and therapies to select which treatments will work best for which patients (NIH, 2015)." This announcement suggests broad support for a move away from viewing victims of disease as members of a homogenous group and towards a future in which treatment of individuals might become the norm. The studies presented in this manuscript represent a small contribution to the growing effort to build the body of scientific evidence necessary to move precision medicine from concept to reality.

REFERENCES

Abecasis, G.R., et al. (2012). An integrated map of genetic variation from 1,092 human genomes. *Nature 491*, 56-65.

Abraham, A. D., Cunningham, C. L., & Lattal, K. M. (2012). Methylphenidate enhances extinction of contextual fear. *Learn Mem*, *19*(2), 67-72.

Adriani, W., Chiarotti, F., & Laviola, G. (1998). Elevated novelty seeking and peculiar damphetamine sensitization in periadolescent mice compared with adult mice. *Behav Neurosci, 112*, 1152–1166.

Agren, T., Engman, J., Frick, A., Bjorkstrand, J., Larsson, E.M., Furmark, T., & Fredrikson, M. (2012). Disruption of reconsolidation erases a fear memory trace in the human amygdala. *Science 337*, 1550-1552.

Altshuler, L.L., Hendrick, V., & Cohen, L.S. (1998) Course of mood and anxiety disorders during pregnancy and the postpartum period. *J Clin Psychiatry*, *59* (Suppl 2), 29-33.

Andersen, S.L. & Teicher, M.H. (2008). Stress, sensitive periods and maturational events in adolescent depression. *Trends in Neurosci*, *31*(4), 183-191.

Baker, K. D., McNally, G. P., & Richardson, R. (2012). d-cycloserine does not facilitate fear extinction by reducing conditioned stimulus processing or promoting conditioned inhibition to contextual cues. *Learn Mem*, *19*(10), 461-469.

Baker, K. D., McNally, G. P., & Richardson, R. (2013). Memory retrieval before or after extinction reduces recovery of fear in adolescent rats. *Learn Mem*, *20*, 467-473.

Baran, S.E., Armstrong, C.E., Niren, D.C., Hanna, J.J., & Conrad, C.D. (2009): Chronic stress and sex differences on the recall of fear conditioning and extinction. *Neurobiol Learn Mem*, *91*, 323-332.

Baran, S.E., Armstrong, C.E., Niren, D.C., & Conrad, C.D. (2010). Prefrontal cortex lesions and sex differences in fear extinction and perseveration. *Learn Mem*, *17*, 267-278.

Bar-Haim, Y., Lamy, D., Pergamin, L., Bakermans-Kranenburg, M. J., & Van Ijzendoorn, M. H. (2007). Threat-related attentional bias in anxious and nonanxious individuals: a meta-analytic study. *Psychol Bull*, *133*(1), 1-24.

Bar-Haim, Y. (2010). Research review: attention bias modification (ABM): a novel treatment for anxiety disorders. *J Chil Psycho Psychi*, *51*(8), 859-870.

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A. R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science*, *269*, 1115e1118.

Beck, A.T. & Emery, G. (1985) *Anxiety disorders and phobias: A cognitive perspective*. New York: Basic Books.

Bennett, K., Manassis, K., Walter, S. D., Cheung, A., Wilansky- Traynor, P., Diaz-Granados, N., ... & Wood, J. J. (2013). Cognitive behavioral therapy age effects in child and adolescent anxiety: An individual patient data metaanalysis. *Dep Anx* , *30*(9), 829-841.

Bennett, S. (November 15, 2014). *Personal communication*, Weill Cornell Medical College.

Berry, A. C., Rosenfield, D., & Smits, J. A. (2009). Extinction retention predicts improvement in social anxiety symptoms following exposure therapy. *Dep Anx, 26*(1), 22-27.

Bisson, J. I., Ehlers, A., Matthews, R., Pilling, S., Richards, D., & Turner, S. (2007) Psychological treatments for chronic post-traumatic stress disorder systematic review and meta-analysis. *British. J. Psych*. *190*, 97-104.

Bramham, C.R. & Messaoudi, E. (2005). BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. *Prog Neurobio*, *76*, 99-125.

Bourgeois, J.P., Goldman-Rakic, P.S., & Rakic, P. (1994). Synaptogenesis in the prefrontal cortex of rhesus monkeys. *Cereb Cort, 4*(1), 78-96.

Bouton, M. (2004). Context and behavioral processes in extinction. *Learn Mem*, *11*(5), 485-494.

Bremner, J.D., Staib, L.H., Kaloupek, D., Southwick, S.M., Soufer, R., & Charney, D.S. (1999). Neural correlates of exposure to traumatic pictures and sound in Vietnam combat veterans with and without posttraumatic stress disorder: a positron emission tomography study. *Bio Psych*, *45*(7), 806-816.

Burghy, C. A., Stodola, D. E., Ruttle, P. L., Molloy, E. K., Armstrong, J. M., Oler, J. A., ... & Birn, R. M. (2012). Developmental pathways to amygdala-prefrontal function and internalizing symptoms in adolescence. *Nat Neuro*, *15*(12), 1736-1741.

Burgos-Robles, A., Vidal-Gonzalez, I., Santini, E., & Quirk, G.J. (2007). Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. *Neuron*, *53*, 871–880.

Burgos-Robles, A., Vidal-Gonzalez, I., & Quirk, G.J. (2009). Sustained conditioned responsesin prelimbic prefrontal neurons are correlated with fear expression and extinction failure. *J Neuro*, *29*, 8474–8482.

Callaghan, B.L. & Richardson, R. (2011). Maternal separation results in early emergence of adult-like fear and extinction learning in infant rats. *Beh Neuro, 125*, 20-28.

Casey, B. J., Galvan, A., & Hare, T. A. (2005). Changes in cerebral functional organization during cognitive development. *Curr Op Neurobio*, *15*(2), 239-244.

Casey B.J., Galvan A., & Getz S. (2008). The adolescent brain. *Dev Rev, 28*(1), 62–77.

Casey, B. J., Jones, R. M., Levita, L., Libby, V., Pattwell, S. S., Ruberry, E. J., ... & Somerville, L. H. (2010). The storm and stress of adolescence: insights from human imaging and mouse genetics. *Dev Psychobio*, *52*(3), 225-235.

CD S. *STAI: manual for the State Trait Anxiety Inventory*, 3 Ed. Consulting Psychologists Press (1988).

Chan, K. H., Morell, J. R., Jarrard, L. E., & Davidson, T. L. (2001). Reconsideration of the role of the hippocampus in learned inhibition. *Beh Brain Res*, *119*(2), 111-130.

Chattopadhyaya, B., Di Cristo, G., Higashiyama, H., Knott, G. W., Kuhlman, S. J., Welker, E., & Huang, Z. J. (2004). Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. *J Neuro*, *24*(43), 9598-9611.

Chhatwal, J.P., Davis, M., Maguschak, K.A., & Ressler, K.J. (2005). Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology, 30*, 516-524.

Chhatwal, J.P., & Ressler, K.J. (2007). Modulation of fear and anxiety by the endogenous cannabinoid system. *CNS Spectr, 12*, 211.

Christianson, J.P., Benison, A.M., Jennings, J., Sandsmark, E.K., Amat, J., Kaufman, R.D., Baratta, M.V., Paul, E.D., Campeau, S., Watkins, L.R., Barth, D.S., & Maier, S.F. (2008). The sensory insular cortex mediates the stress-buffering effects of safety signals but not behavioral control. *J Neuro*, *28*, 13703–13711.

Christianson, J.P., Jennings, J.H., Ragole, T., Flyer, J.G., Benison, A.M., Barth, D.S., Watkins, L.R., & Maier, S.F. (2011) Safety signals mitigate the consequences of uncontrollable stress via a circuit involving the sensory insular cortex and bed nucleus of the stria terminalis. *Biol Psychiatry*, *70*, 458 – 464.

Chiang, K.P., Gerber, A.L., Sipe, J.C., & Cravatt, B.F. (2004). Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. *Hum Mol Genet 13*, 2113-2119.

Chen, Z., Jing, D., Bath, K., Ieraci, A., Khan, T., Siao, C., Herrera, D.G., Toth, M., Yang, C., McEwen, B.S., Hempstead, B.L., & Lee, F.S. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science, 314*, 140-143.

Clem, R. L., & Huganir, R. L. (2010). Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science*, *330* (6007), 1108-1112.

Collins, D.R. & Pare D. (2000). Differential fear conditioning induces reciprocal changes in the sensory responses of lateral amygdala neuron to the CS+ and CS-. *Learn Mem*, *7*, 97-103.

Coppens, E., Spruyt, A., Vandenbulcke, M., Van Paesschen, W., & Vansteenwegan, D. (2009). Classically conditioned fear responses are preserved following unilateral temporal lobectomy in humans when concurrent US-expectancy ratings are used. *Neuropsychologia, 47*, 2496–2503.

Costello, E.J., Egger, H., & Angold, A. (2005). 10-year research update review: the epidemiology of child and adolescent psychiatric disorders. *J Amer Acad Child Adol Psych*, *44*(10), 972-986.

Cravatt, B. F., Demarest, K., Patricelli, M. P., Bracey, M. H., Giang, D. K., Martin, B. R., & Lichtman, A. H. (2001). Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci*, *98*, 9371.

Davidson, J. R., Foa, E. B., Huppert, J. D., Keefe, F. J., Franklin, M. E., Compton, J. S., ... & Gadde, K. M. (2004). Fluoxetine, Comprehensive Cognitive Behavioral Therapy, and Placeboin Generalized Social Phobia. *Arch Gen Psychiatry*, *61*(10), 1005-1013.

Debiec, J., LeDoux, J.E., & Nader, K. (2002). Cellular and systems reconsolidation in the hippocampus. *Neuron*, *36*, 527–538.

Dębiec, J., & Ledoux, J. E. (2004.) Disruption of reconsolidation but not consolidation of auditory fear conditioning by noradrenergic blockade in the amygdala. *Neuroscience, 129*(2), 267-272.

Delgado, M.R., Olsson, A., & Phelps, E.A. (2006). Extending animal models of fear conditioning to humans. *Biol Psychol*, *73*, 39–48

Deschaux, O., Spennato, G., Moreau, J.L., & Garcia, R. (2011). Chronic treatment with fluoxetine prevents the return of extinguished auditory-cued conditioned fear. *Psychopharmacology*, *215*(2), 231–237.

Douglas, R.J. (1967). The hippocampus and behavior. *Psychol Bull*, *67*(6), 416-422.

Doyere, V., Debiec, J., Monfils, M., Schafe, G., and LeDoux, J. (2007). Synapse-specific reconsolidation of distinct fear memories in the lateral amygdala. *Nature*, *10*(4), 414-416.

Dragunow, M., & Faull, R. (1989) The use of c-fos as a metabolic marker in neuronal pathway tracing. *J Neurosci Methods 29*, 261-265.

Drysdale, A., Hartley, C., Pattwell, S., Ruberry, E., Somerville, L., Compton, S., Lee, F.S., Casey, B.J., & Walkup, J. (2013). Fear and anxiety from principle to practice: implications for when to treat youth with anxiety disorders. *Biol Psych, 75*(11), e19-e20.

Dudai, Y. (2006). Reconsolidation: The advantage of being refocused. *Curr Opin Neuro*, *16*(2), 174-178.

Dulawa, S.C., & Hen, R. (2005). Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neurosci Biobehav Rev 29*, 771-783.

Dunsmoor, J.E., Bandettini, P.A., & Knight, D.C. (2008). Neural correlates of unconditioned response diminution during Pavlovian conditioning. *Neuroimage 40,* 811-817.

Duvarci, S., & Nader, K. (2004). Characterization of fear memory reconsolidation. *J Neuro*, *24*(42), 9269-9275.

Eelen, P., & Vervliet, B. (2006). *Conditioning and anxiety: an historical perspective. In Fear and Learning: From Basic Processes to Clinical Implications*. Washington, DC: Am. Psychol. Assoc.

Egan, M.F., Kojima, M., Callicot, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D.R. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257-269.

Ettenberg, A., & Carter, H. (1986). Haloperidol induces a partial reinforcement extinction effect in rats: implications for a dopamine involvement in food reward. *Pharm Bio Beh,* 25(4), 813-821.

Eysenck, H. J. (1952). The effects of psychotherapy: an evaluation. *J Consult Psych*, *16*(5), 319.

Eysenck H.J. (1960) *Handbook of abnormal psychology: An experiential approach* (pp. 675-725). London: Pitman Medical.

Felmingham, K.L., Dobson-Stone, C., Schofield, P.R., Quirk, G., & Bryant, R.A. (2013). The brain-derived neurotrophic factor val66met polymorphism predicts response to exposure therapy in posttraumatic stress disorder. *Biol Psych*, *73*(11), 1059-1063.

Fredrikson, M., & Öhman, A. (1979). Cardiovascular and Electrodermal Responses Conditioned to Fear- Relevant Stimuli. *Psychophysiology, 16*, 1-7.

Funayama, E.S., Grillon, C., Davis, M., & Phelps, E.A. (2001). A double dissociation in the affective modulation of startle in humans: effects of unilateral temporal lobectomy. *J Cog Neuro, 13*, 721–729.

Galvan A, et al. (2006) Earlier development of the accumbens relative to orbitofrontal cortex might underlie risk-taking behavior in adolescents. *J Neuro*, *26*, 6885–6892.

Gee, D.G., Humphreys, K.L., Flannery, J., Goff, B., Telzer, E.H., Shapiro, M., Hare, T.A., Bookheimer, S.Y., & Tottenham, N. (2013). A developmental shift from positive to negative connectivity in human amygdala-prefrontal circuitry. *J Neuro*, *33*(10), 4584–4593.

Gewirtz, J. C., Falls, W. A., & Davis, M. (1997). Normal conditioning inhibition and extinction of freezing and fear-potentiated startle following electrolytic lesions of medial prefrontal cortex in rats. *Beh Neuro*, *111*(4), 712.

Gogtay, N., Giedd, J. N., Lusk, L., Hayashi, K. M., Greenstein, D., Vaituzis, A. C., ... & Thompson, P. M. (2004). Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Nat Acad Sci USA*, *101*(21), 8174-8179.

Goldstein, J.M., Seidman, L.J., Horton, N.J., Makris, N., Kennedy, D.N., Caviness, V.S., Faraone, S.V., Tsuang, M.T. (2001). Normal sexual dimorphism of the adult

human brain assessed by in vivo magnetic resonance imaging. *Cereb Cortex*, *11*, 490-497.

Golkar, A., Bellander, M., Olsson, A., & Ohman, A. (2012). Are fear memories erasable? -reconsolidation of learned fear with fear-relevant and fear irrelevant stimuli. *Front Beh Sci*, *6*(80), 1-10.

Gottfried, J. A., & Dolan, R. J. (2004). Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nat Neuro*, *7*(10), 1144-1152.

Graff, J., Joseph, N.F., Horn, M.E., Samiei, A., Meng, J., Seo, J., Rei, D., Dero, A., Phan, T., Wagner, F., Holson, E., Xu, J., Sun, J., Neve, R., Mach, R., Haggarty, S.J., & Tsai, L. (2014). Epigenetic priming or memory updating during reconsolidation to attenuate remote fear memories. *Cell, 156*, 261-276.

Graham, B. M., & Richardson, R. (2009). Acute systemic fibroblast growth factor-2 enhances long-term extinction of fear and reduces reinstatement in rats. *Neuropsychopharmacology*, *34*(7), 1875-1882.

Green, J.G., McLaughlin, K.A., Berglund, P.A., Gruber, M.J., Sampson, N.A., Zaslavsky, A.M. & Kessler, R.C. (2010). Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: Associations with first onset of DSM-IV disorders. *Arch Gen Psych*, *67*(2), 113–123.

Greenberg, P. E., Sisitsky, T., Kessler, R. C., Finkelstein, S. N., Berndt, E. R., Davidson, J. R., ... & Fyer, A. J. (1999). The economic burden of anxiety disorders in the 1990s. *J Clin Psych*, *60*, 427-435.

Gross, J. J. (2001). Emotion regulation in adulthood: Timing is everything. *Curr Dir Psych Sci*, *10*(6), 214-219.

Guastella, A. J., Lovibond, P. F., Dadds, M. R., Mitchell, P., & Richardson, R. (2007). A randomized controlled trial of the effect of D-cycloserine on extinction and fear conditioning in humans. *Beh Res Ther*, *45*(4), 663-672.

Gunduz-Cinar, O. et al. (2013a). Convergent translational evidence of a role for

anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. *Mol Psych, 18*, 813.

Gunduz-Cinar, O., Hill, M.N., McEwen, B.S., & Holmes, A.H. (2013b) Amygdala FAAH and anandamide: mediating protection and recovery from stress. *Trends Pharmacol Sci 34*, 637.

Gunnar, M.R., Bruce, J., & Grotevant, H.D. (2000). International adoption of institutionally reared children: research and policy. *Dev Psychpathology, 12*, 677-693.

Gunnar, M.R., & van Dulmen, M.H. (2007). Behavior problems in postinstitutionalized internationally adopted children. *Dev Psychopathology, 19*(1), 129–148.

Gutman, A. R., Yang, Y., Ressler, K. J., & Davis, M. (2008). The role of neuropeptide Y in the expression and extinction of fear-potentiated startle. *J Neuro*, *28*(48), 12682-12690.

Guyer, A.E., Monk, C.S., McClure-Tone, E.B., Nelson, E., Roberson-Nay, R., Adler, A.D., Fromm, S.J., Leibenluft, E., Pine, D.S. & Ernst, M. (2008). A developmental examination of amygdala response to facial expressions. *J Cog Neuro, 20*(9), 1565-1582.

Haaker, J., Gaburro, S., Sah, A., Gartmann, N., Lonsdorf, T. B., Meier, K., ... & Kalisch, R. (2013). Single dose of L-dopa makes extinction memories context-independent and prevents the return of fear. *Proc Nat Acad Sci U S A*, *110*(26), E2428-E2436.

Haddad, A. D. M., Lissek, S., Pine, D. S., & Lau, J. Y. F. (2011). How do social fears in adolescence develop? Fear conditioning shapes attention orienting to social threat cues. *Cog and Emo*, *25*(6), 1139-1147.

Hahn, A., Stein, P., Windischberger, C., Weissenbacher, A., Spindelegger, C., Moser, E., ... & Lanzenberger, R. (2011). Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. *Neuroimage*, *56*(3), 881-889.

Hakamata, Y., Lissek, S., Bar-Haim, Y., Britton, J. C., Fox, N. A., Leibenluft, E., ... & Pine, D. S. (2010). Attention bias modification treatment: a meta-analysis toward the establishment of novel treatment for anxiety. *Bio Psych*, *68*(11), 982-990.

Hare, T.A., Tottenham, N., Galvan, A., Voss, H., Glover, G. & Casey, B.J. (2008). Biological substrates of emotional reactivity and regulation in adolescent during an emotional go-nogo task. *Bio Psych*, *63*, 927-934.

Harsh, V., Meltzer-Brody, S., Rubinow, D.R., & Schmidt, P.J. (2009). Reproductive aging,sex steroids, and mood disorders. *Harv Rev Psych*, *17*, 87-102.

Hariri, A. R., Gorka, A., Hyde, L. W., Kimak, M., Halder, I., Ducci, F., ... & Manuck, S. B. (2009). Divergent effects of genetic variation in endocannabinoid signaling on human threat-and reward-related brain function. *Bio Psych*, *66*(1), 9-16.

Hartley, C.A., & Phelps, E.A. (2010). Changing fear: the neurocircuitry of emotion regulation. *Neuropsychopharmacology, 35*, 136-146.

Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. *Nat Rev Neurosci, 6*, 877–888.

Hermans, D., Dirikx, T., Vansteenwegenin, D., Baeyens, F., Van den Bergh, O., & Eelen, P. (2005). Reinstatement of fear responses in human aversive conditioning. *Beh Res Ther*, *43*, 533-551.

Hettema, J. M., Annas, P., Neale, M. C., Kendler, K. S., & Fredrikson, M. (2003). A twin study of the genetics of fear conditioning. *Arch Gen Psych, 60,* 702-708.

Hill, M.N., Miller, G.E., Ho, W.S., Gorzalka, B.B., & Hillard, C.J. (2008). Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. *Pharmacopsychiatry, 41*, 48-53.

Hofer, M.A. (1996). On the nature and consequences of early loss. *Psychosomatic Med 58*(6), 570–581.

Hughes, K.C., & Shin, L.M. (2011). Functional neuroimaging studies of posttraumatic stress disorder. *Expert Rev Neurother, 11*, 275–285.

Huttenlocher, P. R., & Dabholkar, A. S. (1997). Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neuro*, *387*(2), 167-178.

Indovina, I., Robbins, T.W., Nunez-Elizalde, A.O., Dunn, B.D., & Bishop, S.J. (2011). Fear-conditioning mechanisms associated with trait vulnerability to anxiety in humans. *Neuron*, *69*(3), 563-571.

Jenkins, W. O., & Stanley Jr, J. C. (1950). Partial reinforcement: a review and critique. *Psych Bul*, *47*(3), 193.

Johnson, D.C., & Casey, B.J. (2015a). Easy to remember, difficult to forget: the development of fear regulation. *Dev Cog Neuro*, *11*, 42-55.

Johnson, D.C. & Casey, B.J. (2015b). Extinction during memory reconsolidation blocks recovery of fear in adolescents. *Sci Rep*, *5*, 8863, DOI:10.1038/srep08863.

Jovanovic, T., Norrholm, S. D., Fennell, J. E., Keyes, M., Fiallos, A. M., Myers, K. M., ... & Duncan, E. J. (2009). Posttraumatic stress disorder may be associated with impaired fear inhibition: relation to symptom severity. *Psych Res*, *167*(1), 151-160.

Jovanovic, T., Norrholm, S.D., Blanding, N.Q., Davis, M., Duncan, E., Bradley, B., & Ressler, K.J. (2010a). Impaired fear inhibition is a biomarker of PTSD but not depression. *Dep Anx*, *27*, 244e251.

Jovanovic, T., Norrholm, S. D., Blanding, N. Q., Phifer, J. E., Weiss, T., Davis, M., ... & Ressler, K. (2010b). Fear potentiation is associated with hypothalamic–pituitary–adrenal axis function in PTSD. *Psychoneuroendocrinology*, *35*(6), 846-857.

Jovanovic, T., Kazama, A., Bachevalier, J., & Davis, M. (2012). Impaired safety signal learning may be a biomarker of PTSD. *Neuropharmacology*, *62*(2), 695-704.

Kalisch, R., Korenfeld, E., Stephan, K. E., Weiskopf, N., Seymour, B., & Dolan, R. J. (2006). Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. *J Neuro*, *26*(37), 9503-9511.

Karpova, N. N., Pickenhagen, A., Lindholm, J., Tiraboschi, E., Kulesskaya, N., Ágústsdóttir, A., ... & Castrén, E. (2011). Fear erasure in mice requires synergy between antidepressant drugs and extinction training. *Science, 334*(6063), 1731-1734.

Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., ... & Piomelli, D. (2003). Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med*, *9*(1), 76-81.

Kazama, A., Heuer, E., Davis, M., & Bachevalier, J. (2010). Long-term effects of selectiveneonatal lesions of the amygdala, hippocampus, or areas 11 & 13 of the orbitofrontalcortex on fear regulation. In: Society for Neuroscience Meeting, San Diego, CA

Keller, M. B., Lavori, P. W., Wunder, J., Beardslee, W. R., Schwartz, C. E., & Roth, J. (1992). Chronic course of anxiety disorders in children and adolescents. *J Amer Acad Child Adol Psych*, *31*(4), 595-599.

Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R., & Walters, E.E. (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Arch Gen Psych*, *62*, 593– 602.

Kilb, W. (2012). Development of the GABAergic system from birth to adolescence. *The Neuroscientist*, *18*(6), 613-630.

Kim, H., Somerville, L., Johnstone, T., Polis, S., Alexander, A., Shin, L., & Whalen, P. (2004). Contextual modulation of amygdala responsivity to surprised faces. *J Cog Neuro 16*(10), 1730-1745.

Kim, J.M., & Whalen, P.J. (2009). The structural integrity of an amygdala-prefrontal pathway predicts trait anxiety. *J Neuro*, *29*(3), 11614-11618.

Kim, M.J., Gee, D.G., Loucks, R.A., Davis, F.C., Whalen, P.J. (2011a). Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. *Cereb Cortex, 21*, 1667-1673.

Kim, M. J., Loucks, R. A., Palmer, A. L., Brown, A. C., Solomon, K. M., Marchante, A. N., & Whalen, P. J. (2011). The structural and functional connectivity of the amygdala: from normal emotion to pathological anxiety. *Beh Brain Res*, *223*(2), 403-410.

Kim, J.H., Li, S., & Richardson, R. (2011). Immunohistochemical analyses of long-term extinction of conditioned fear in adolescent rats. *Cer Cort*, *21*, 530-538.

Kim-Cohen, J., Caspi, A., Moffitt, T.E., Harrington, H., Milne, B.J., & Poulton, R. (2003). Prior juvenile diagnoses in adults with mental disorder: developmental follow-back of a prospective-longitudinal cohort. *Arch of Gen Psych*, *60*, 709-717.

Kimble, D.P. (1968). Hippocampus and internal inhibition. *Psych Bull, 70*(5), 285-295.

Kindt, M., Soeter, M., & Vervliet, B. (2009). Beyond extinction: erasing human fear responses and preventing the return of fear. *Nat Neuro, 12*(3), 256-258.

Kindt, M., & Soeter, M. (2013). Reconsolidation in a human fear conditioning study: a test of extinction as updating mechanism. *Bio Psych*, *92*(1), 43-50.

LaBar, K.S., Gatenby, J.C., Gore, J.C., LeDoux, J.E., & Phelps, E.A. (1998). Human amygdala activation during conditioned fear acquisition and extinction: a mixed-trial fMRI study. *Neuron, 20*, 937-945.

Lach, G., & de Lima, T. C. M. (2013). Role of NPY Y1 receptor on acquisition, consolidation and extinction on contextual fear conditioning: dissociation between anxiety, locomotion and non-emotional memory behavior. *Neuro Learn Mem*, *103*, 26-33.

LeDoux, J. (2000). Emotion circuits in the brain. *Ann Rev Neuro*, *23*, 155-184.

Lang, P. J., Bradley, M.M. & Cuthbert, B.N. (2005). International affective picture system (IAPS): Affective ratings of pictures and instruction manual. NIMH, Center for the Study of Emotion & Attention.

Lattal, K. M., Barrett, R. M., & Wood, M. A. (2007). Systemic or intrahippocampal delivery of histone deacetylase inhibitors facilitates fear extinction. *Beh Neuro*, *121*(5), 1125.

Laurent, V., & Westbrook, R.F. (2009). Inactivation of the infralimbic but not the prelimbic cortex impairs consolidation and retrieval of fear extinction. *Learn Mem, 16*, 520-529.

Laviola, G., Adriani, W., Terranova, M.L., & Gerra, G. (1999). Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models. *Neuro Bio Rev*, *23*, 993–1010.

Leaton, R.N., & Cranney, J. (1990). Potentiation of the acoustic startle response by a conditioned stimulus paired with acoustic startle stimulus in rats. *J Exp Psychol Anim Behav Process, 16*(3): 279–87.

LeDoux, J. (2003). The emotional brain, fear and the amygdala. *Cell Mol Neuro*, *23*(4-5), 727-738.

LeDoux, J. (2007). The Amygdala. *Curr Biol*, *17*, R868-874.

Lee, J. L., Milton, A. L., & Everitt, B.J. (2006). Reconsolidation and extinction of conditioned fear: inhibition and potentiation. *J Neuro*, 26(39), 10051-10056.

Lévesque, J., Joanette, Y., Mensour, B., Beaudoin, G., Leroux, J. M., Bourgouin, P., & Beauregard, M. (2004). Neural basis of emotional self-regulation in childhood. *Neuroscience*, *129*(2), 361-369.

Levita, L., Hare, T.A., Voss, H.U., Glover, G., Ballon, D.J. & Casey, B.J. (2009). The bivalent side of the nucleus accumbens. *Neuroimage, 44*, 1178–87.

Lewis, D. J. (1960). Partial reinforcement: a selective review of the literature since 1950. *Psychol Bull, 57*, 1-28.

Liberman, L.C., Lipp, O.V., Spence, S.H., & March, S. (2006). Evidence for retarded extinction of aversive learning in anxious children. *Behav Res Ther*, *44*, 1491–1502.

Likhtik, E., Pelletier, J.G., Paz, R., & Pare, D. (2005) Prefrontal control of the amygdala. *J Neuro*, *25*, 7429–7437.

Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., & Pine, D.S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. *Beh Res Ther*, *43*(11), 1391–1424.

Liu, J., Zhao, L., Xue, Y., Shi, J., Suo, L., Luo, Y., ... & Lu, L. (2014). An unconditioned stimulus retrieval extinction procedure to prevent the return of fear memory. *Biol Psych*, *76*(11), 895-901.

Mahan, A.L., & Ressler, K.J. (2012). Fear conditioning, synaptic plasticity and the amygdala: implications for posttraumatic stress disorder. *Trends Neuro*, *35*(1), 24-35.

Mackintosh, N.J. (1974). The Psychology of Animal Learning. London: Academic Press.

MacLeod, C., & Mathews, A. (2012). Cognitive bias modification approaches to anxiety. *Ann Rev Clin Psych*, *8*, 189-217.

Malter-Cohen, M., Jing, D., Yang, R.R., Tottenham, N., Lee, F.S., & Casey, B.J. (2013). Early-life stress has persistent effects on amygdala function and development in mice and humans. *Proc Nat Acad Sci USA*, *110*(45), 18274-18278.

Maren, S. (2001). Neurobiology of Pavlovian fear conditioning. *Ann Rev Neuro*, *24*, 897-931.

Mathews, A., & MacLeod, C. (1985). Selective processing of threat cues in anxiety states. *Beh Res Ther*, *23*(5), 563-569.

McCallum J., Kim J.H., & Richardson, R. (2010). Impaired extinction retention in adolescent rats: effects of D-cycloserine. *Neuropsychopharmacology*, *35*, 2134-2142.

McGaugh, J.L. (2000). Memory–a century of consolidation. *Science*, *287*(5451), 248-251.

McNally, R. J. (2007). Mechanisms of exposure therapy: How neuroscience can improve psychological treatments for anxiety disorders. *Clin Psych Rev*, *27*, 750-759.

McRae, K., Gross, J. J., Weber, J., Robertson, E. R., Sokol-Hessner, P., Ray, R. D., . . . & Ochsner, K. N. (2012). The development of emotion regulation: An fMRI study of cognitive reappraisal in children, adolescents and young adults. *Soc Cog Affect Neuro*, *7*(1), 11–22.

Merikangas, K.R., He, J.P., Burstein, M., Swanson, S.A., Avenevoli, S., Cui, I., Benjet, C., Georgiades, K., Swendsen, J. (2010). Lifetime prevalence of mental disorders in U.S. adolescents: results from the National Comorbidity Survey Replication – Adolescent supplement (NCS-A). *J Amer Acad Child Adol Psych*, *49*, 980-989.

Milad, M.R., & Quirk, G.J. (2002). Neurons in medial prefrontal cortex signal memory for fear extinction. *Nature*, *420*, 70–74.

Milad, M. R., Orr, S. P., Pitman, R. K., & Rauch, S. L. (2005). Context modulation of memory for fear extinction in humans. *Psychophysiology*, *42*(4), 456-464.

Milad, M. R., Quirk, G. J., Pitman, R. K., Orr, S. P., Fischl, B., & Rauch, S. L. (2007). A role for the human dorsal anterior cingulate cortex in fear expression. *Biol Psych*, *62*(10), 1191-1194.

Milad, M. R., Wright, C. I., Orr, S. P., Pitman, R. K., Quirk, G. J., & Rauch, S. L. (2007b). Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. *Biol Psych* 62(5), 446-454.

Milad, M.R., Orr, S.P., Lasko, N.B., Chang, Y., Rauch, S., & Pitman, R.K. (2008). Presence and Acquired Origin of Reduced Recall for Fear Extinction in PTSD: Results of a Twin Study. *J Psych Res*, *42*(7), 515-520.

Milad, M. R., Pitman, R. K., Ellis, C. B., Gold, A. L., Shin, L. M., Lasko, N. B., ... & Rauch, S. L. (2009). Neurobiological basis of failure to recall extinction memory in posttraumatic stress disorder. *Biol Psych*, *66*(12), 1075-1082.

Milad, M.R., Igoe, S.A., Lebron-Milad, K., & Novales, J.E. (2009). Estrous cycle phase andgonadal hormones influence conditioned fear extinction. *Neuroscience*, *164*, 887-895.

Milad, M.R., Zeidan, M.A., Contero, A., Pitman, R.K., Klibanski, A., Rauch, S.L., & Goldstein, J.M. (2010). The influence of gonadal hormones on conditioned fear extinction in healthy humans. *Neuroscience, 168*, 652-658.

Milad, M.R., & Quirk, G.J., (2012). Fear extinction as a model for translational neuroscience: ten years of progress. *Ann Rev Psych, 63*, 129-151.

Mills, K. L., Goddings, A. L., Clasen, L. S., Giedd, J. N., & Blakemore, S. J. (2014). The developmental mismatch in structural brain maturation during adolescence. *Dev Neuro*, *36*(3–4), 147-160.

Mineka, S., & Ohman, A. (2002). Phobias and preparedness: the selective, automatic, and encapsulated nature of fear. *Biol Psych*, *52*(10), 927–37.

Misanin, J. R., Miller, R. R., & Lewis, D. J. (1968). Retrograde amnesia produced by electroconvulsive shock after reactivation of a consolidated memory trace. *Science*, *160* (3827), 554-555.

Mobbs, D., Yu, R., Rowe, J. B., Eich, H., Feldman-Hall, O., & Dalgleish, T. (2010). Neural activity associated with monitoring the oscillating threat value of a tarantula. *Proc Nat Acad Sci U S A*, *107*(47), 20582-20586.

Mogoaşe, C., David, D., & Koster, E. H. (2014). Clinical Efficacy of Attentional Bias Modification Procedures: An Updated Meta- Analysis. *J Clin Psych*, *70*(12), 1133-1157.

Monfils, M. H., Cowansage, K. K., Klann, E., & LeDoux, J. E. (2009). Extinction-reconsolidation boundaries: key to persistent attenuation of fear memories. *Science, 324* (5929), 951-955.

Monk, C.S., McClure, E.B., Nelson, E.E., Zarahn, E., Bilder, R.M., Leibenluft, E., Charney, D.S., Ernst, M., & Pine, D.S. (2003). Adolescent immaturity in attention-related brain engagement to emotional facial expressions. *Neuroimage,* 20, 420-428.

Monk, C. S., Telzer, E. H., Mogg, K., Bradley, B. P., Mai, X., Louro, H. M., ... & Pine, D. S. (2008). Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. *Arch Gen Psych*, *65*(5), 568-576.

Moreira, F.A., Kaiser, N., Monory, K., & Lutz, B. (2008). Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by CB1 receptors. *Neuropharmacology, 54*, 141.

Myers, K.M., & Davis, M. (2002). Behavioral and neural analysis of extinction. *Neuron 36*, 567-584.

Nader, K., Schafe, G.E., & LeDoux, J.E. (2000). Fear memories require protein synthesis in the amygdala for reconsolidation after retrieval. *Nature*, *406*, 722-726.

Nelson, C.A., Zeanah, C.H., Fox, N.A., Marshall, P.J., Smyke, A.T., & Guthrie, D. (2007). Cognitive recovery in socially deprived young children: the Bucharest Early Intervention Project. *Science*, *318*(5858), 1937–1940.

Newman, D. L., Moffitt, T. E., Caspi, A., Magdol, L., Silva, P. A., & Stanton, W. R. (1996). Psychiatric disorder in a birth cohort of young adults: Prevalence, comorbidity, clinical significance, and new case incidence from ages 11–21. *J Consulting Clin Psych*, *64*(3), 552.

Nieuwenhuys, R. (2012). The insular cortex: a review. *Prog Brain Res, 195*, 123–163.

NIH. (2015). http://www.nih.gov/precisionmedicine. Accessed February 13, 2015.

Ochsner, K. N., Ray, R. D., Cooper, J. C., Robertson, E. R., Chopra, S., Gabrieli, J. D., & Gross, J. J. (2004). For better or for worse: neural systems supporting the cognitive down-and up-regulation of negative emotion. *Neuroimage*, *23*(2), 483-499.

Ohman, A., & Mineka, S., 2001. Fears, phobias and preparedness: toward an evolved module of fear and fear learning. *Psych Rev, 108*(3), 3483-522.

Oyarzun, J.P., Lopez-Barroso, D.L., Fuentemilla, L., Cucurell, D., Pedraza, C., Rodriguez-Fornells, A., & Diego-Balaguer, R. (2012). Updating fearful memories with extinction training during reconsolidation: A human study using auditory aversive stimuli. *PLOS One, 7*(6), e38849.

Paulus, M.P., Stein, M.B. (2006). An insular view of anxiety. *Biol Psych*, *60*, 383–387

Paré, D., Quirk, G.J., Ledoux, J.E. (2004). New vistas on amygdala networks in conditioned fear. *J Neurophysiol, 92*, 1–9.

Pattwell, S.S., Bath, K.G., Casey, B.J., Ninan, I., & Lee, F.S. (2011). Selective early-acquired fear memories undergo temporary suppression during adolescence. *Proc Nat Acad Sci U S A*, *108*, 1182–1187.

Pattwell, S., Duhoux, S., Hartley, C.A., Johnson, D.C., Jing, D., Elliott, M.D., Ruberry, E.J., Powers, A., Mehta, N., Yang, R.R., Soliman, F., Glatt, C.E., Casey, B.J., Ninan, I., & Lee, F.S. (2012a). Altered fear learning across development in both mouse and human. *Proc Nat Acad Sci U S A*, *109*(40), 16318-16323.

Pattwell, S. S., Bath, K. G., Perez-Castro, R., Lee, F. S., Chao, M. V., & Ninan, I. (2012b). The BDNF Val66Met polymorphism impairs synaptic transmission and plasticity in the infralimbic medial prefrontal cortex. *J Neuro Sci*, *32*(7), 2410-2421.

Pattwell, S. S., Lee, F. S., & Casey, B. J. (2013). Fear learning and memory across adolescent development: Hormones and Behavior Special Issue: Puberty and Adolescence. *Hormones Beh*, *64*(2), 380-389.

Pavlov, I.P., & Anrep, G.P. (1927). Conditioned Reflexes: An Investigation of the Physiological Activity of the Cerebral Cortex. Oxford University Press/Humphrey Milford, London.

Peters, J., Dieppa-Perea, L.M., Melendez, L.M., & Quirk, G.J. (2010). Induction of fear extinction with hippocampal-infralimbic BDNF. *Science, 328*, 1288-1290.

Petersen, A.C., Crockett, L., Richards, M. & Boxer, A. (1988). A self-report measure of pubertal status: Lessons from research on anxiety. *J Youth Adol* 17, 117–133.

Phelps, E.A., Delgado, M.R., Nearing, K.I., & LeDoux, J.E. (2004). Extinction learning in humans: role of the amygdala and vmPFC. *Neuron, 43*, 897-905.

Phelps, E.A., & LeDoux, J.E. (2005). Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron, 48*, 175-187.

Pitman, R.K., Sanders, K.M., Zusman, R.M., Healy, A.R., Cheema, F., Lasko, N.B., Cahill, L., & Orr, SP. (2002). Pilot study of secondary prevention of posttraumatic stress disorder with propranolol. *Biol Psych, 51*(2), 189-192.

Pollack, M. H., Otto, M. W., Sabatino, S., Majcher, D., Worthington, J. J., McArdle, E. T., & Rosenbaum, J. F. (1996). Relationship of childhood anxiety to adult panic disorder: correlates and influence on course. *Amer J Psychiatry*, *153*(3), 376-381.

Quinn, R. (2005). Comparing rat's to human's age: How old is my rat in people years? *Nutrition*, 21, 775–777.

Quirk, G.J., Repa, C., & LeDoux, J.E. (1995). Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. *Neuron*, *15*(5), 1029-1039.

Quirk, G.J., Garcia, R., & González-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biol Psychiatry, 60*, 337–343.

Quirk, G.J., & Mueller, D. (2008). Neural mechanisms of extinction learning and retrieval. *Neuropsychopharmocology Rev*, *33*, 56-72.

Rakofsky, J.J., Ressler, K.J., & Dunlop, B.W. (2012). BDNF function as a potential mediator of bipolar disorder and post-traumatic stress disorders co-morbidity. *Mol Psychiatry, 17*, 22-35.

Repa, J. C., Muller, J., Apergis, J., Desrochers, T. M., Zhou, Y., & LeDoux, J. E. (2001). Two different lateral amygdala cell populations contribute to the initiation and storage of memory. *Nat Neuro*, *4*(7), 724-731.

Rescorla, R.A. (1969). Pavlovian conditioned inhibition. *Psychological Bull*, *72*(2), 77.

Rescorla, R.A., & Wagner, A.R. (1972) A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. In A.H. Black & W.F. Prokasy (Eds.), Classical Conditioning II: Current research and theory (pp. 64-99). New York: Appleton-Century-Crofts.

Ressler, K. J., Rothbaum, B. O., Tannenbaum, L., Anderson, P., Graap, K., Zimand, E., ... & Davis, M. (2004). Cognitive enhancers as adjuncts to psychotherapy: Use of D-cycloserine in phobic individuals to facilitate extinctionof fear. *Arch Gen Psychiatry*, *61*(11), 1136-1144.

Robbins, D. (1971). Partial reinforcement: a selective review of the alleyway literature since 1960. *Psychol Bull, 76*, 415-431.

Rodgers, K.M., Benison, A.M., Klein, A., & Barth, D.S. (2008) Auditory, somatosensory, and multisensory insular cortex in the rat. *Cereb Cortex, 18*, 2941–2951.

Romeo, R. D., Mueller, A., Sisti, H. M., Ogawa, S., McEwen, B. S., & Brake, W. G. (2003). Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. *Hormones Beh*, *43*(5), 561-567.

Rothbaum, B.O., & Davis, M. (2003). Applying learning principles to the treatment of post-trauma reactions. *Ann New York Acad Sci*, *1008*, 112-121.

Rogan, M.T., Leon, K.S., Perez, D.L., & Kandel, E.R. (2005). Distinct neural signatures for safety and danger in the amygdala and striatum of the mouse. *Neuron, 46*, 309–320.

Romeo, R.D., Mueller, A., Sisti, H.M., Ogawa, S., McEwen, B.S., & Brake, W.G., (2003). Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. *Hormones Beh*, *43*, 561–567.

Rutter, M., & O'Connor, T.G. (2004). Are there biological programming effects for psychological development? Findings from a study of Romanian adoptees. *Dev Psychology*, *40*(1), 81–94.

Rutter, M., Sonuga-Barke, E. J., Beckett, C., Castle, J., Kreppner, J., Kumsta, R., ... &

Gunnar, M. R. (2010). Deprivation-Specific Psychological Patterns: Effects of Institutional Deprivation. *Mono Soc Res Child Dev*, *75*(1), 1-252.

Sangha, S., Chadick, J. Z., & Janak, P. H. (2013). Safety encoding in the basal amygdala. *J Neuro*, *33*(9), 3744-3751.

Santini, E., Quirk, G.J., & Porter, J.T. (2008). Fear conditioning and extinction differentially modify the intrinsic excitability of infralimbic neurons. *J Neuro*, *28*(15), 4028-4036.

Santini, E., & Porter, J. T. (2010). M-type potassium channels modulate the intrinsic excitability of infralimbic neurons and regulate fear expression and extinction. *J Neuro*, *30*(37), 12379-12386.

Sara, S. J. (2010). Reactivation, retrieval, replay and reconsolidation in and out of sleep: connecting the dots. *Front Beh Neuro*, 4.

Schafe, G.E., Nadel, N.V., Sullivan, G.M., Harris, A., & LeDoux, J.E. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA and MAP kinase. *Learn Mem*, *6*(2), 97-110.

Schafe, G. E., Doyère, V., & LeDoux, J. E. (2005). Tracking the fear engram: the lateral amygdala is an essential locus of fear memory storage. *J Neuro*, *25*, 10010-10014.

Schiller, D., Levy, I., Niv, Y., LeDoux, J.E., & Phelps, E.A. (2008). From fear to safety and back: Reversal of fear in the human brain. *J Neuro*, *28*, 11517–11525.

Schiller, D., Monfils, M.H., Raio, C.M., Johnson, D.C., LeDoux, J.E., & Phelps, E.A., (2010a). Preventing the return of fear in humans using reconsolidation update mechanisms. *Nature*, *463* (7277), 49-53.

Schiller, D., & Delgado, M.R. (2010b) Overlapping neural systems mediating extinction, reversal and regulation of fear. *Trends Cogn Sci, 14*, 268-276.

Schiller, D., & Phelps, E.A. (2011). Does reconsolidation occur in humans? *Front Beh Neuro*, *5*(24), 1-11.

Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M. H., & Phelps, E. A. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *Proc Nat Acad Sci U S A*, *110*(50), 20040-20045.

Schmidt, P.J., & Rubinow, D.R. (2009). Sex hormones and mood in the perimenopause. *Ann N Y Acad Sci*, *1179*, 70-85.

Schnatz, P.F., Whitehurst, S.K., O'Sullivan, D.M. (2010). Sexual dysfunction, depression, and anxiety among patients of an inner-city menopause clinic. *J Women's Health*, *19*, 1843-1849.

Semple, W. E., Goyer, P. F., McCormick, R., Compton-Toth, B., Morris, E., Donovan, B., ... & Schulz, S. C. (1996). Attention and regional cerebral blood flow in posttraumatic stress disorder patients with substance abuse histories. *Psychiatry Res Neuroimaging*, *67*(1), 17-28.

Sevenster, D., Beckers, T., & Kindt, M. (2012). Retrieval per se is not sufficient to trigger reconsolidation of human fear memory. *Neurobiology Learn Mem*, *97*, 338-345.

Shiban, Y., Brütting, J., Pauli, P., & Mühlberger, A. (2015). Fear reactivation prior to exposure therapy: Does it facilitate the effects of VR exposure in a randomized clinical sample? *J Beh Ther Exp Psych*, *46*, 133-140.

Shin, L. M., McNally, R. J., Kosslyn, S. M., Thompson, W. L., Rauch, S. L., Alpert, N. M., ... & Pitman, R. K. (1999). Regional cerebral blood flow during script-driven imagery in childhood sexual abuse-related PTSD: a PET investigation. *Amer J Psychiatry, 156* (4), 575-584.

Shin, L.M., Rauch, S.L., & Pitman, R.K. (2006). Amygdala, medial prefrontal cortex, and hippocampal function in PTSD. *Ann New York Acad Sci*, 1071, 67-79.

Shonkoff, J. P., Boyce, W. T., & McEwen, B. S. (2009). Neuroscience, molecular biology, and the childhood roots of health disparities: building a new framework for health promotion and disease prevention. *J Amer Med Assn*, *301*(21), 2252-2259.

Sierra-Mercado, D., Padilla-Coreano, N., & Quirk, G.J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology, 36*, 529-538.

Silvers, J. A., McRae, K., Gabrieli, J. D., Gross, J. J., Remy, K. A., & Ochsner, K. N. (2012). Age-related differences in emotional reactivity, regulation, and rejection sensitivity in adolescence. *Emotion*, *12*(6), 1235.

Sipe, J.C., Chiang, K., Gerber, A.L., Beutler, E., & Cravatt, B.F. (2002) A missense mutation in human fatty acid amide hydrolase associated with problem drug use. *Proc Natl Acad Sci U S A, 99*, 8394.

Sipe, J. C., Scott, T. M., Murray, S., Harismendy, O., Simon, G. M., Cravatt, B. F., & Waalen, J. (2010). Biomarkers of endocannabinoid system activation in severe obesity. *PLoS One*, *5*(1), e8792.

Sierra-Mercado, D., Padilla-Coreano, N., & Quirk, G.J. (2011). Dissociable roles of prelimbic and infralimbic cortices ventral hippocampus and basolateral amygdala in the expression and extinction of conditioned fear. *Neuropsychopharmacology, 36*(2), 529-538.

Soeter, M. & Kindt, M. (2011). Disrupting reconsolidation: pharmacological and behavioral manipulations. *Learn Mem, 18*(6): 357–66.

Soliman, F., Glatt, C., Bath, K., Levita, L., … & Casey, B.J. (2010). A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science*, *327* (5967), 863-866.

Sotres-Bayon, F., & Quirk, G.J. (2010). Prefrontal control of fear: more than just extinction. *Curr Op Neurobiology, 20*, 231-235.

Sotres-Bayon, F., Sierra-Mercado, D., Pardilla-Delgado, E., & Quirk, G.J. (2012). Gating of fear in prelimbic cortex by hippocampal and amygdala inputs. *Neuron*, 76 (4), 804-812.

Sowell, E. R., Thompson, P. M., Holmes, C. J., Jernigan, T. L., & Toga, A. W. (1999). In vivo evidence for post-adolescent brain maturation in frontal and striatal regions. *Nature Neuro*, *2*(10), 859-861.

Sowell, E. R., Thompson, P. M., Leonard, C. M., Welcome, S. E., Kan, E., & Toga, A. W. (2004). Longitudinal mapping of cortical thickness and brain growth in normal children. *J Neuro*, *24*(38), 8223-8231.

Spear, L.P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci Biobehav Rev, 24*, 417–463.

Spielberger, C.D., et al. (1983) *Manual for the State-Trait Anxiety Inventory* (Consulting Psychologists Press, Palo Alto).

Squire, L.R., & Davis, H.P. (1981). The pharmacology of memory: a neurobiological perspective. *Ann Rev Pharm Tox*, *21*, 323-356.

Squire, L. R., & Knowlton, B. J. (2000). The medial temporal lobe, the hippocampus, and the memory systems of the brain. New Cog Neuro, *2*, 756-776.

Steinfurth, E. C., Kanen, J. W., Raio, C. M., Clem, R. L., Huganir, R. L., & Phelps, E. A. (2014). Young and old Pavlovian fear memories can be modified with extinction training during reconsolidation in humans. *Learn Mem*, *21*(7), 338-341.

Tanner, J.M. *Growth at Adolescence* (Blackwell Scientific Publication, Oxford, 1962).

Tottenham, N., Hare, T.A., Quinn, B.T., McCarry, T.W., Nurse, M., Gilhooly, T., Millner, A., Galvan, A., Davidson, M.C., Eigsti, I., Thomas, K.M., Freed, P.J., Booma, E.S., Gunnar, M.R., Altemus, M., Aronson, J., & Casey, B.J. (2010). Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci*, *13*(1), 46-61.

Urry, H. L., Van Reekum, C. M., Johnstone, T., Kalin, N. H., Thurow, M. E., Schaefer, H. S., ... & Davidson, R. J. (2006). Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *J Neuro*, *26*(16), 4415-4425.

Vaiva, G., Ducrocq, F., Jezequel, K., Averland, B., Lestavel, P., Brunet, A., & Marmar, C.R. (2003). Immediate treatment with propranolol decreases posttraumatic stress disorders two months after trauma. *Biol Psychiatry*, *54*(9), 947-949.

Vidal-Gonzalez, I., Vidal-Gonzalez, B., Rauch, S.L., Quirk, G.J. (2006). Microstimulation reveals opposing influences of prelimbic and infralimbic cortex on the expression of conditioned fear. *Learn Mem 13*, 728-733.

Verma, D., Tasan, R. O., Herzog, H., & Sperk, G. (2012). NPY controls fear conditioning and fear extinction by combined action on Y1 and Y2 receptors. *Brit J Pharmacology*, *166*(4), 1461-1473.

Vertes, R.P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse, 51*, 32–58.

Vertes, R.P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience, 142*, 1–20.

Vyas, A., Mitra, R., Shankaranarayana Rao, B.S., & Chattarji, S. (2002). Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neuro*, *22*(15), 6810–6818.

Walkup, J.T., Albano, A.M., Piacentiti, J., Birmaher, B., Compton, S.N., Sherrill, J.T., Ginsburg, G., Rynn, M.A., McCracken, J., Waslick, B., Lyengar, S., March, J.S., & Kendall, P.C. (2008). Cognitive behavioral therapy, sertraline or a combination in childhood anxiety. *New Eng J Med*, *359*, 2573-2766.

Wang, S.H., de Oliveira, A. L., & Nader, K. (2009). Cellular and systems mechanisms of memory strength as a constraint on auditory fear reconsolidation. *Nat Neuro*, *12*(7), 905–912.

Watson, J.B., & Rayner, R. (1920). Conditioned emotional reactions. *J Exp Psychology*, *3*, 1-14.

Weike, A. I., Schupp, H. T., & Hamm, A. O. (2007). Fear acquisition requires awareness in trace but not delay conditioning. *Psychophysiology*, *44*, 170–180.

Wolpe, J. (1958) Psychotherapy by reciprocal inhibition. Stanford, CA: Stanford University Press.

Xue, Y.-X., Luo, Y.-X., Wu, P., Shi, H.-S., Xue, L.-F., Chen, C., . . . & Lu, L. (2012). A memory retrieval-extinction procedure to prevent drug craving and relapse. *Science, 336*, 241–245.

Zeidan, M.A., Igoe, S.A., Linnman, C., Vitalo, A., Levine, J.B., Klibanski, A., Goldstein, J.M., Milad, M.R. (2011). Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. *Biol Psychiatry*, 2011, 70:920-927.

Zhang, L., Benedek, D.M., Fullerton, C.S., Forsten, R.D., Naifeh, J.A., Li, X.X., Hu, X.Z., Li, H., Jia, M., Xing, G.Q., Benevides, K.N., & Ursano, R.J. (2014). PTSD risk is associated with BNDF val66met and BDNF overexpression. *Mol Psychiatry*, *19*, 8-10.